THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

NORTH OF ENGLAND SECTION

THE tenth Summer Meeting was held at the Prince of Wales Hotel, Scarborough, from June 9th to 12th. The attendance, which included many ladies, was sixty.

The Chairman (Professor T. P. Hilditch) presided, and among those present were the following:—President (Professor W. H. Roberts); Past-Presidents: Dr. Bernard Dyer with Mrs. Dyer, Mr. J. Evans, Mr. E. Hinks with Mrs. Hinks, and Dr. G. Roche Lynch with Miss Roche Lynch; Honorary Secretary (Mr. L. Eynon); Honorary Treasurer (Dr. E. B. Hughes with Mrs. Hughes); Scottish Section, Honorary Secretary (Mr. J. B. McKean); Miss Bradford; Mr. R. C. Chirnside; Dr. H. E. Cox; Mr. F. W. Edwards and Mrs. Edwards; Mr. E. M. Hawkins; Mr. F. G. H. Tate and Mrs. Tate; Scottish Section: Mr. A. R. Jamieson.

The Chairman gave a welcome to all members, particularly those from the South and from Scotland, and also referred to the great loss sustained by the Society since the last Summer Meeting by the death of Dr. J. T. Dunn and Mr. E. R. Bolton.

On Saturday morning a paper was read by G. W. Monier-Williams, O.B.E., M.C., M.A., Ph.D., F.I.C., on "Lead in Food." A discussion followed the paper, and a resolution of thanks, proposed by Mr. S. E. Melling and seconded by Mr. A. R. Jamieson, was unanimously passed to Dr. Monier-Williams for contributing the paper.

A resolution expressing the greetings of the Section and declaring its loyalty to the Council and the Parent Society was proposed by Mr. J. G. Sherratt, seconded by Mr. F. Robertson Dodd, and was passed unanimously.

On Sunday afternoon a motor run was made over the moors towards Whitby, and tea was taken at the Goathland Hydro.

Grateful acknowledgment is made by the Honorary Secretary, Mr. J. R. Stubbs, to the following for their services in carrying out the arrangements:—Mr. W. G. Carey, Mr. A. Lees, Mr. T. W. Lovett, and Mr. F. J. Smith.

Biological Standardisation

By Sir HENRY DALE, C.B.E., M.D., F.R.S.

(*Address given at the Annual General Meeting, March 3rd,* 1939)

There are many important medicinal substances and preparations for which the ordinary chemical and physical methods of analysis are unable to give the data required to standardise them. Many of them have powerful, definite and therapeutically important activities, making them directly dangerous in some cases if they are given in excess. It is accordingly a matter of practical urgency that preparations of such substances should be standardised to a constant strength, specified within narrow limits, or should have their potency indicated in some clear and accepted notation. The latter is much the more desirable aim. It enables dosage to be given in real equivalents in practical medicine, and, what is even more important, it provides a common basis for the expression and record of the results of research, which has often been greatly hampered and retarded by the lack of it. The principles responsible, in such cases, for the specific actions are as yet unknown, or, if known, cannot for one reason or another be measured by chemical or physical methods. The only property by which such an active principle can be recognised and measured is its action on the living animal or surviving organ, which must somehow be made the basis of what is called a "biological standardisation" or "biological assay."

You will probably have observed that it is in relation to the more recently introduced types of remedial agents that such methods have found their chief application. Such newer remedies have this in common, that they aim at dealing with the causes of disease, by directly replacing defects, or directly reinforcing natural functions or defensive reactions, and not merely at alleviating or palliating symptoms, as do so many of the older medicines, as well as their modern, synthetic substitutes. The use of natural remedies dealing with causes—the antitoxins and immunological remedies, the hormones and the vitamins—is a relatively modern development. It began to become effective only a little before the opening of the present century, and has therefore a history of only some 40 or 50 years, in comparison with the age-long use of the unnatural medicines modifying symptoms, the origin of which goes back to the very beginnings of recorded history. Practically none of these natural remedies, when they first come under investigation and are first applied, can be assayed by methods of ordinary analysis. In one case or another, indeed, the progress of research, promoted by a common notation of biological activity, has succeeded in isolating the active hormone or vitamin, and has thereby enabled an adequate chemical method for its measurement to be devised, or has even led to the synthetic production of the pure principle, and the use of this in medicine, in place of a crude extract requiring standardisation. But though such chemical progress has been astonishingly rapid in recent years, even more rapid has been the emergence of newly discovered principles with great remedial potentialities, which, for the time at least, must have their activities measured by biological methods and recorded in a biological notation. For the present, then, and for some time to come, we shall have to regard methods of biological

assay as necessary for a large, and probably a growing section of practical therapeutics, and for some of the most progressive departments of research in pathology, physiology and biochemistry.

DEVELOPMENT OF BIOLOGICAL ASSAY.—I propose briefly to review the history of the method, to glance at the nature and origin of some of the misunderstandings that have arisen with regard to it, and to trace the emergence of the principles which have enabled it to acquire a sounder scientific reputation, and to provide the basis for a world-wide uniformity of notation for a number of important remedies.

While, of course, some crude kind of measurement of the effect of drugs upon men and animals has existed since medicine began, and was given a greater precision when experimental pharmacology began to record the smallest doses of various drugs that would produce recognisable degrees of action, the earliest case known to me of the employment of an action on living animals as a basis for the indication of a dosage in units of activity, instead of in units of weight or measure, was that of the antitoxic serum obtained by immunising an animal, usually a horse, by injections of the diphtheria toxin. We know now that it differs from the normal serum in the proportion between the globulins and the albumins making up its proteins. For some years past it has been possible to detect the proportion of antitoxin which just neutralises a given toxin by observing the earliest appearance of an aggregation or precipitate in a series of such mixtures; and certain very recent developments have enabled the antitoxic moiety of the globulin to be split off by controlled and restricted enzymatic hydrolysis, and thus obtained in a more definite form. There are encouraging signs, therefore, that it may ultimately be possible to determine the specific activity of this and similar preparations by purely physical and chemical methods. At the time of its discovery, however, and for many years afterwards, there was no method known by which the presence of the antitoxin in a serum could even be detected, much less its quantity measured, except by testing its power of neutralising the action of the toxin on an animal sensitive to it. In France the attempt was made to do this by determining the dose needed to save an animal from an actual infection by diphtheria bacilli; in Germany they tried to do it by finding the dose of a serum which would neutralise a certain number of lethal doses—so-called—of a toxin. The results obtained on these lines were so confusing and misleading, that it required a research which is one of the classics of immunology, and, indeed, provided the basis for all true biological standardisation, to bring order out of the muddle. This was due to the genius of Paul Ehrlich, who, having thus put the assay of antitoxic sera on a sound basis, and opened new fields to exploration in the science of immunology, turned his attention to the systematic search for artificial synthetic remedies for infections, discovered salvarsan, and inaugurated the new science of specific chemotherapy, which, as yet, has only begun to show its great potentialities.

Ehrlich must have observed that the term "minimal lethal dose" of a toxin had in itself no precise meaning—a point to which we shall later return. The point, however, of special interest and importance was his discovery that two toxins of equal toxic potency, as nearly as this could be measured, might require widely different proportions of the same antitoxin to neutralise their toxic actions.

He found the explanation for this anomaly in the fact that every sample of toxin contained a variable proportion of material which had lost its toxic action, but retained its affinity for antitoxin. He saw, then, that the only way to order and safety, out of this confusion, lay in the establishment of a fixed, stable, standard sample of completely desiccated antitoxin, sealed up in dry vacuum, and kept in cold storage. The unit of antitoxin was defined, once for all, as the neutralising or binding-power of a given weight of this dried serum sample. The test dose of any toxin was the smallest amount which, when combined with this unit, would leave sufficient toxin free to kill a guinea-pig in a standard period of 4 days; and, when the test dose of a particular toxin had so been determined, the volume of any serum containing one unit could be ascertained by mixing a close series of dilutions, each with the test dose of toxin, and finding which mixture left just enough toxin free to kill a guinea-pig in the standard time.

There is one point about this and similar methods, in which the unit of a serum neutralises a large number of lethal doses of a toxin, which deserves special notice. Let us suppose that the number of binding doses of a particular toxin which the serum just neutralises is 1000. Then, theoretically, the test dose of the toxin contains 1000 binding doses + 1 lethal dose; but you will see that the accuracy of the determination would not be seriously affected if the dose actually determined left free 5 lethal doses instead of the theoretical single lethal dose, the difference of 4 lethal doses involving an error of only a very small percentage of the total number involved in the test. Under such conditions the variations in the sensitiveness of the different guinea-pigs in a test series, inevitable even when their ages, weights and all other conditions are kept constant, become practically of small account. The guinea-pigs are simply indicators in a titration, and the fact that their sensitiveness may vary over a certain range introduces an error not seriously greater than that caused in acidimetry by the addition of phenolphthalein, for example, in an amount not accurately measured. The point has real importance, because I strongly suspect that the accuracy obtainable in such an antitoxin titration, even with only one or two guinea-pigs on each mixture, led to the erroneous assumption, from false analogy, that the single lethal dose of an unmixed poison could be determined with comparable accuracy, with the use of one, or at most a few animals on each dose. It took, in fact, a good deal of work and advocacy at a later stage to eliminate this delusion, and to convince those concerned that the expression "lethal dose," or "minimal lethal dose," had no real meaning. The point, however, which I wish specially to emphasise, as established by Ehrlich's work on antitoxin standardisation, is that a biological assay or standardisation, like any other standardisation or measurement, must be comparative, and made in relation to a fixed and invariable standard of reference.

Ehrlich had unfortunately used the term "minimal lethal dose" in connection with his antitoxin standardisation, but had escaped the misleading assumption that the phrase implies, because, as I have suggested, he was determining only the end-point of a titration. This was not the case with the many attempts to measure the activity of toxic or physiologically active substances directly, in terms of a lethal effect, or other limiting animal reaction. The first instance of this kind was the attempt to standardise preparations of digitalis, by determining the dose required to

stop a frog's heart in permanent contraction. One might suppose, from some of the writings on the subject, and the frequent reference to "frog units," that there was a sharp line, or at most a narrow margin, between the dose per gramme of body weight from which every frog would recover, and the dose per gramme that would kill every frog receiving it. Such an idea would, of course, be very far from the truth. I believe that Greenwood was the first to point out, in a study of figures obtained by Rowland for the lethal effect of different doses of a plague-bacillus toxin on rats, that there was a wide range of doses, within which the question whether a particular animal would die or survive was a matter not of certainty, but only of statistically calculable chance. A good many years later Trevan, in a very valuable and effective paper, clearly indicated that the term "minimal lethal dose," which had become so prevalent in biological literature, had no clear or proper meaning. It might mean, either the smallest dose capable of killing even one animal of a very large number receiving it, or the smallest dose which would not allow even one to survive. Though in certain rare cases these two values might be fairly close together, in most cases, and in all those for which a biological standardisation had been found necessary, there was a wide disparity, the certainly lethal dose being several times, and often many times, that which would still kill occasionally. These doses at the extremes of the whole lethal range, moreover, were not capable of exact determination, the curves relating dose to proportionate lethality being S-shaped, and becoming asymptotic to the abscissa at each end. The range in which the dose producing a certain effect can be measured with most precision is not at the ends of the curve, but at the middle point, where the dose kills about one-half of the animals injected. We ought, therefore, as Trevan suggests, to speak, not vaguely of a "minimal lethal dose," but of a dose lethal for a stated percentage of animals, choosing for assay purposes a dose falling on the steepest part of the graph relating mortality to dosage. Obviously, on a limited number of animals, even this dose cannot be determined exactly, but only within a certain range of probable error. We can experimentally determine the "standard deviation," and calculate the frequency with which the result will have a given degree of inaccuracy. We can thus make allowance for any desired approach to certainty, but no more. The cold blast of statistics has had a bracing effect, and has swept away a good deal of flimsy assumption with which the subject was cumbered. It is of curious interest to recall how the number 3 seemed, at one time, to have a mysterious attraction or significance for workers in this field. We were recommended, for example, to find the dose of a digitalis preparation which would kill 2 out of 3 frogs injected, and to regard this as the lethal dose; it seemed to give to some an added confidence, if such a result was stated as "a 66·6 *per cent. mortality*." Later, we were recommended to test a selected dose of an insulin preparation on rabbits until we found three consecutive rabbits giving the same fall of blood sugar, and then to accept this result as giving a true measure of the activity; a method, as I once remarked, for which the only recorded authority was the Bellman in *The Hunting of the Snark*, with his pronouncement that "What I tell you three times is true."

PRINCIPLES OF BIOLOGICAL STANDARDISATION.—Gradually, by the application of sound reasoning and statistical analysis, the subject has been lifted above such

facile assumptions and credulous optimism, and we know much more clearly how the nearest approach to quantitative accuracy can be secured, and, in each particular case, how wide the limit of inevitable error remains. Some years ago I ventured to put forward certain general principles, to which any biological standardisation ought to conform. I would maintain them still, with modifications only of the details of their application in particular cases.

(1) Like any other standardisation or measurement, it is essentially comparative, and must be made in relation to a fixed, material standard. Apart from individual variations among a common stock of a species of animal, the sensitiveness of a whole stock may vary in unknown ways and to unknown degrees with season, temperature, diet or other unrecognised conditions. There can be no such thing as a frog, mouse, or rabbit unit; the animal can only serve as a reagent in a strictly comparative test.

(2) The standard chosen must owe the activity, which the test measures, to the active principle for which the preparation is to be assayed.

(3) Provided the biological reaction used in the test affords a means of measuring a known active principle with sufficient accuracy, it need have no relation to the therapeutic effect. It has often been suggested, quite absurdly, that the comparative assay of digitalis by use of the permanent stoppage of a frog's heart as an end-point reaction must give results having no relation to its therapeutic value, because digitalis is used in medicine to strengthen the hearts of men and not to stop those of frogs. It would be as rational to argue that a colorimetric assay, which also depends, in the reading, on a biological reaction, can have no value, because the drug assayed is not used in medicine to produce a colour.

(4) The best type of test, when it is available, is one in which the standard and the preparation under test are compared in succession on the *same* animal or preparation. Individual variation is thus completely eliminated, and we have only to allow for a change of sensitiveness with the progress of the experiment, which can be discounted by alternation of dosage.

(5) Usually, however, no such method can be used. We often have to depend on the observation of what my friend Prof. Gaddum has termed a "quantal" response—the presence or absence of some recognisable, limiting response in each of a series of animals. It is then that we have to introduce statistical considerations, and to calculate, in each case, the number of animals on a dose which will suffice to enable us to determine the strength, with sufficient accuracy for practical purposes, by observation of the proportion showing the "quantal" response. In other instances, we observe the degree of a measurable effect—such as a gain in weight on a vitamin dosage, or a fall in blood sugar produced by insulin. In such cases also we must use sufficient numbers to reduce the error due to individual variations, and we must, as always, give the test a comparative basis by simultaneous measurement of the effect of a known number of units of the standard preparation, upon a strictly comparable group of animals from the same stock, and subject to identical conditions. When the effect is evanescent, as that of the fall of blood sugar produced by insulin, we can

eliminate individual idiosyncrasy, though not the day-to-day variation shown by a single individual. This is effected by using the same two groups of animals on two occasions, at a suitable interval, giving group A the standard and group B the sample under test on the first occasion, and reversing the order on the second; so that the response of every animal in both groups, to both standard and sample, can be used in comparative calculation of the strength of the sample.

The most fundamental of these principles, applying to every case, is that the standardisation is made by a comparative test, in relation to a fixed standard, in terms of which the unit of activity is once for all defined. The unit is the fixed value, which must remain unchanged even when a new standard has to be made for replacement, or because a better material for it becomes available. Such a system keeps the unit fixed, and provides, in the material standard, a currency for its transmission all over the world. Under these conditions, as the unit becomes generally adopted, the results of scientific work in all countries become comparable by the use of a common quantitative notation, and the greatest freedom can be allowed in experiment directed to the improvement of existing methods, and the discovery of better ones, for applying the common standard in comparative measurements.

INTERNATIONAL STANDARDS.—These principles have provided the basis of action in the creation, since 1921, of a large series of International Standards for therapeutic agents, the activity of which can only be determined by comparative biological tests. At various stages, and in relation to successive substances of this kind, it has been necessary to stand firmly for the principle of fixed material standards, and units defined in terms of them, and to resist the common tendency of investigators to invent and promulgate units of their own, defined in terms of some animal reaction which they have found it convenient to observe. Time and again, when the question of international action for the standardisation of some substance has arisen, it has been found that the subject is already encumbered and common action compromised by the existence, for the one substance, of a whole series of different and incommunicable units, promulgated by different investigators, each basing his definition on some animal reaction which he has found convenient, and which, with a constancy of conditions unattainable outside his own laboratory and his own stock of animals, he believes that he can measure with a sufficient uniformity. In repeated instances it has become obvious that, even with animals of the same stock, but kept in different laboratories, small variations in age, diet, prevalent temperature and other conditions may lead to widely different values for a unit, if it is thus defined in terms of an animal reaction. And in every case the only way to uniformity has been found in the creation of a common material standard, and the definition in terms of it of a common unit, independent of the nature of the test chosen for its comparative measurement.

The international work began in 1921 in connection with the Health Organisation of the League of Nations, on the initiative of Dr. Thorwald Madsen, Director of the State Serum Institute, Copenhagen. Before the war the whole world had depended for samples of the standard for diphtheria antitoxin on the goodwill of Ehrlich's own Institute in Frankfurt, where the original standard of dried serum

had been adopted and was preserved. Subsidiary standards, with unit values determined by exact comparison with Ehrlich's, had fortunately been set up in a number of other countries, and particularly, on a national basis, in the Public Health Department at Washington. Otherwise the outbreak of war, shutting off most of the world from access to the original, basic standard kept in Frankfurt, might have led to confusion and discrepancy between the units used in different countries for measuring and indicating the activity of this important remedy. With the object of discovering the extent to which such discrepancies had, in fact, arisen, and of providing against the recurrence of such uncertainties, the first International Conference dealing with such matters met in London in 1921, on the initiative of the Health Organisation of the League of Nations, and with Dr. Madsen as President. It is worthy of notice, I think, that this Conference had interest and value apart from its result in recorded and effective agreement. It provided, I believe, the first occasion for the meeting, in friendly discussion, with the express object of practical agreement on a scientific matter, of experts from the different countries so recently at war; for, like all the subsequent Conferences having a similar purpose, the experts invited to attend it were chosen on a scientific and not on a political basis, and without any reference to membership of the League. It is much to be regretted that in recent years a new, political attitude has intruded, through the refusal of countries which have withdrawn from the League to allow their experts to continue to participate in such scientific activities for the common good.

The first Conference, in 1921, accepted Ehrlich's unit for dipheria antitoxin, and arranged for the production of a new, international standard preparation, in terms of which that unit could be re-defined. This is now deposited, for custody and distribution, in Dr. Madsen's Institute, the State Serum Institute, Copenhagen. The second International Conference, under the same auspices, met in Paris in 1922, to deal similarly with tetanus antitoxin. They adopted as a basis the American Standard, but, unfortunately, not without a gratuitous change from the American value in defining a unit for international use. This was a rare example of the intrusion of other than strictly scientific considerations into this international activity; and it has contributed to the difficulties which have left the basis of the standardisation of this important serum still, at the present moment, short of a final, international agreement.

A little later Dr. Madsen, whose own special field is immunology, approached me concerning the possibility of doing something towards international uniformity in the standardisation of remedies of other kinds—drugs, hormones and others—for which biological methods were required and had been applied. It seemed possible that we might do something about digitalis and its allies, and about hormone preparations, such as those of the pituitary posterior lobe and the then newly-discovered insulin. It was obvious that something ought to be done, if possible, for the position was no better than chaotic. My then colleague, Dr. Burn, now Professor of Pharmacology at Oxford, had been conducting with me a survey of the activities of the different preparations of the posterior-pituitary extract obtainable in this country; we had found that, in respect of the activity as a stimulant of the uterus to contraction, for which the extract was widely applied in

obstetrics, the different preparations varied over such a range that the strongest had 80 times the activity of the weakest. Even in one country, then, preparations all labelled "physiologically standardised," and offered for use, mostly in emergency, with the same dosage, could be completely ineffective at one end of the scale, and strong enough to threaten rupture of the labouring uterus at the other. As for insulin, with its peculiar need for accurate adjustment of dose, in that a relatively small excess might produce a dangerous condition while a small under-dosage would cause relapse, already some three different rabbit units and a mouse unit had been proposed, and nobody knew clearly their relation to one another, even if any of them had been accurately and consistently measurable in different laboratories. The travelling victim of diabetes had a poor prospect, indeed, of obtaining the immense benefits of this new treatment with safety and regularity, as he wandered from one country to another. There was clearly an urgent call for prompt international action in these cases, and an international Conference of experts met in Edinburgh in 1923, and took the first steps towards it. Digitalis and its allies, pituitary posterior lobe extract and insulin were the chief subjects of discussion, with side-glances at the possibility of action in other directions. After some three days of discussion, most vigorous and obstinate in the case of insulin, the Conference unanimously agreed to organise and carry out co-operative tests of the suitability of dry, stabilised standards for these three main items of our discussion. The late Prof. Magnus of Utrecht undertook to prepare such a standard for digitalis, and Prof. Voegtlin of Washington accepted the responsibility for the pituitary standard. When the advocacy of rival animal units for insulin had reduced their activities to exhaustion and deadlock, the members of the Conference also agreed to consider the definition of a common unit, independent of methods, in terms of a dry, stable standard preparation of insulin, though they were sceptical as to the possibility of making one. I was in the fortunate position of being able to state with confidence that, if we were given the supplies of insulin, my late colleague, Dr. H. W. Dudley, had a method ready for making the stable standard. When the same group of experts, with others added to their number, met again for a fuller discussion at Geneva, in 1925, the data for agreement were all to hand, as the result of really international, co-operative trials in the intervening years.

Insulin Standard.—Everybody concerned was ready to ignore fractional discrepancies, and to agree forthwith that the new standard for insulin should be regarded as having 8 *units of activity in* 1 *milligramme.* Thereafter it did not take long to secure the adoption, throughout the world, of one common unit of insulin, as thus defined. Dudley's standard preparation, then adopted, remained current and authoritative till 1935. By that time the late J. J. Abel's success in obtaining insulin in the form of crystals, and the later discovery by Scott, of Toronto, that the crystals were a zinc salt of insulin, had made it possible to obtain crystalline insulin, as a pure and regularly reproducible product, in any amount required. The original, impure standard being then within measurable distance of exhaustion, the opportunity was taken to make a new standard of the crystalline substance, in sufficient quantity to last for very many years; and the unit value of this was again determined in a number of institutions in several countries, by elaborate, comparative assay, in terms of the original standard of 1925. There followed a

good deal of discussion as to the precise unit value to be assigned to the new standard, ending in agreement to regard it as containing 22 *units per milligramme*; and on that basis the unit of insulin ought now to be fixed for all time, or for so long as insulin preparations still need to be assayed by biological methods.

Pituitary Posterior Lobe Extract Standard.—For this extract the Conference of 1925 readily accepted the dry standard, but spent much time, unwisely as it seemed to me, in trying to find an agreed strength, in terms of that standard, for a watery extract to be used in all countries and for all purposes. Immediately we came up against the differences of practice and experience in different countries. It appeared that the surgeons and obstetricians of one country would use with cheerful confidence doses which those of other countries regarded as desperately dangerous. Here again, we found a way out, and a basis for ready agreement, in the definition of a unit in terms of the new standard, leaving it a matter of choice, in different countries, to inject ten or two of such units for the same purpose, in accordance with the varying standards of caution and courage in the practitioners, and of sensitiveness or toughness in the patients. Again, therefore, the notation, though not the practice, of the whole world was unified. The original definition of this unit had reference particularly to the activity of the pituitary extract forming the basis of its chief use, in obstetrics. It remained only for later conferences to define similar units, in terms of the same standard, for the other principles contained in this extract, acting on the circulation and on the excretory function of the kidney. These units, I hope, will now stand so long as these pituitary principles require biological assay. Arrangements are now in prospect for replacing the standard, of which a large part has been used, by a new one, in terms of which these units will have to be separately re-defined, without any change in the value of any, so far as careful comparison by a number of experts can avoid it.

Digitalis Standard.—Although the standard sample of digitalis was readily accepted, the conservative attitude of the pharmacologists from most countries did not, at the 1925 Conference, allow them to accept immediately the idea of a unit of activity for so old-fashioned a remedy. They preferred to aim at enforcing a conformity, in the activity of all digitalis used in medicine, to the strength of the accepted standard, by judicious blending of strong and weak samples. To me this procedure always seemed more suitable to the grocery or the spirit trade than to scientific medicine, and a later Conference was persuaded to define a unit of activity for digitalis also. The advantage of this was manifest in 1935, when it became necessary, in due course, to introduce a new and more carefully prepared standard to replace the original one, which had been rather rapidly exhausted by the demand. It was much easier and better to decide, on the basis of co-operative, international comparisons, *that the unit, defined as the activity of* 100 *milligrammes of the old standard, was represented by* 80 *milligrammes of the new one*, than it would have been to hunt for inactive or spoiled samples of the drug, in the hope that these, by suitable blending, would reduce the activity of the new and more perfect material to that of the original standard. The British Pharmacopoeia, which had led the way by adopting the unit basis for digitalis in its 1932 issue, was able to adjust its indications to the new standard by a simple numerical change, made in the corrective Addendum issued in 1936.

Standards for Synthetic Organic Compounds.—The Conference of 1925 had under consideration a group of remedies of a different kind, with regard to which it had been feared that any effort to create international standards would encounter unusual difficulties of national prestige and commercial jealousy. This was a group of synthetic organic compounds containing arsenic, including Ehrlich's salvarsan, with neosalvarsan and certain other derivatives. These were, in theory, known chemical substances, and there could be no question of standardising them in biological units. Their physical properties, however, made it impossible to prepare them pure, and small variations in process had been found to make them, on the one hand, dangerously toxic, or, on the other hand, weak in therapeutic action. It was necessary to provide standard samples, by comparison with which agreed maxima of toxicity and minima of potency could be determined; and when it came to the point, the fears of international acrimony proved to be ill-founded, and standards were readily adopted, which, in spite of the new political difficulties of recent years, are still maintained with the co-operation of the laboratory from which these substances originated.

Extensions of the System of International Standards.—I have spoken in some detail of these early activities in the creation of international standards, not because their direct results were more important than many others that have followed, but because they were effective in establishing traditions and principles on which subsequent action has been based. I must be content with a more rapid review of these later extensions of the system of international standards, to other members of those groups of remedies with which a beginning was made in these earlier years, and to other groups which only later came under effective consideration. Standards for a number of antitoxic and other curative sera have, at several stages, been added to the list, and, by reference to the units based on these, it has been possible secondarily to standardise certain antigens used for the production of an active immunity. The desirability of unification, by international agreement, of the units used in measuring different types of *vitamin activity* was already under discussion at the Geneva Conference in 1925, but only in the sense of an attempt, fortunately abortive, to obtain international recognition for certain detailed methods of testing. The time was not ripe for effective action concerning these important, protective constituents of natural foods, till 1931, when a first International Vitamin Conference, under the auspices of the Health Section of the League of Nations, met in London under the chairmanship of Sir Edward Mellanby. They had to deal with a position largely analogous to that which had earlier been encountered with regard to other classes of remedies—a multiplicity of rat, pigeon or guinea-pig units, each claiming a special convenience, or special accuracy for a particular criterion or end-point, but all subject to the widest variations with small changes of conditions, and none affording any basis of comparison between the values obtained for the same vitamin by the different methods. And here, again, the only basis for agreement and unification was found in the acceptance of stable standards, in terms of which it was easy to define units, which would remain valid and unvaried whatever the tests used for their comparative estimation in relation to the standards. In several instances later Conferences, or agreements by correspondence among members of earlier ones, have been able to substitute better

vitamin standards, consisting of pure, synthetic substances, for the original standards made of the crude materials available at the time of their adoption; but all these new standards have been very carefully evaluated in terms of the old, and the units re-defined so as to involve the smallest unavoidable change of value with change of standard. As an example of the scale of accuracy attainable in such work, I might mention the recent adoption of pure, synthetic *Aneurin* as the international standard for vitamin B_1, in place of the crude adsorption product which formed the original standard. The international comparisons of these old and new standards were made by a whole range of different methods, both prophylactic and curative, on rats and pigeons. Under such a wide variety of conditions, it seemed certain that the comparisons would be affected, in widely different degrees, by differences in the rates of absorption of the vitamin, when given, respectively, as a pure substance and as a crude extract adsorbed on clay. Nevertheless, in the results from all sources and by all methods, the extreme values assigned to the unit were 2γ *and* $3{\cdot}5\gamma$ *of the new, pure standard*, and the large majority were round about 3γ, which was also the precise average of the whole series. There was no difficulty in adopting 3γ, by unanimous agreement, *as the quantity of the new standard containing the unit.* And this new definition, being in terms of a chemically pure substance, should now stand for all time, or for as long as this vitamin continues to be measured in units of activity.

Standardisation of Sex Hormones.—Precisely the same principles have been followed, I am bound to say with less practical and lasting success, in adopting standards and defining units for the sex hormones. We adopted a standard of pure oestrone for oestrogenic action, and defined a unit in terms of it, in the hope of saying good-bye to mouse-units and rat-units. Then came *oestradiol*, and the discovery that attempts to assay it in terms of oestrone units gave widely different results on different species, and with different methods of administration. The ideal of a single standard and unit, independent of method, would not hold as between these different forms of the hormone. It would have been easy to conclude that we must have a different standard for each form; but, on the one hand, it was never possible to know in what relative proportions they would be present in a crude extract, and, on the other hand, manufacturers, with a perverse enterprise and ingenuity, were soon busy with the production of artificial esters, with which the variations of unitage in terms of the original hormone, produced by different methods of administration to different kinds of animal, became even more fantastic; we certainly could not produce a new international standard for every new proprietary ester. Much the same is happening in connection with the male hormones, *androsterone* and *testosterone*, and their artificial esters. When we take note, in addition, of the recent appearance of relatively simple and easily made synthetic substances, like the so-called *stilboestrol*, closely similar in their action to the natural hormones, but stronger, I think we may reasonably forecast a fairly early disappearance of biological standards and units from this particular field, and its replacement by an ordinary dosage in terms of exact weights of pure substances. And I think that all the signs point to a similar change in the field of the vitamins, where identification as pure substances, and the production of these by artificial synthesis, are now proceeding so rapidly as to overtake and outpace

the description of new vitamins. The death-rate, so to speak, of vitamins identifiable only by letters of an alphabet threatened with exhaustion, is at last happily in excess of the birth-rate.

In both these fields, then, we may be able to foresee the end of biological standardisation—a consummation, I venture to say, devoutly to be wished. Meanwhile, however, I would also venture to claim that, even in these fields, the precision given to biological measurement and the comparability of the results from different laboratories, obtained by the establishment of fixed international standards and units, has been a powerful factor in the rapid advance of true chemistry in these fields, and thus in the accelerated progress here of biological standardisation towards its proper goal of self-extinction. The international units will further have provided, at least, a bridge by which the mind of the practical physician, never eager to jump a step in calculation, may be able to pass over the gap between dosage of crude extracts in units and dosage of pure substances in milligrammes. Meanwhile the work of unification is advancing into other fields, and only last summer a Conference at Geneva adopted and defined four new standards, and units in terms of them, for two complex, and, hitherto, chemically intangible hormones of the anterior pituitary lobe, and two analogous principles formed, during pregnancy, in the blood serum and the urine of certain animal species. These are now in course of preparation, and will, in due course, be ready for addition to the already quite imposing series of standards, with corresponding units, which international action has produced.

The following table includes a complete list of the International Standards hitherto adopted and gives an indication of the weight of each representing one unit, and of their distribution, for custody and administration, between the Copenhagen and Hampstead Institutes.

ORGANISATION OF STANDARDISATION.—From the first the International Standards for antitoxic and other sera and immunological remedies have been committed to the care of the State Serum Institute at Copenhagen. My own early association with the international work on the standards for drugs and hormones led to the custody of these being entrusted to the National Institute at Hampstead. In due course the Institute came to be regarded as a kind of international "Pooh-Bah" in these matters, with a responsibility for "everything else" in the way of standards, other than the serum standards. The Therapeutic Substances Act of 1925 brought under State control in this country, for the first time, the production and standardisation of all the sera, salvarsan (arsphenamine) and its derivatives, insulin and post-pituitary extract, and laid on the National Institute the responsibility for preparing and providing national standards, based in all cases on those which had been internationally adopted. We were ready to fulfil this obligation before the Act became effective in 1927. For some of the sera subsidiary standards had to be prepared, and the units defined in relation to them, by exact comparison with the International Standards. For others we ourselves prepared the first standards, of which portions were internationally accepted, and transferred to Copenhagen for international use. In the preparation of the salvarsan standards we had co-operated with Ehrlich's Institute, the Georg Speyer

Haus at Frankfurt, and the preparation of the insulin and pituitary standards had been in our hands from the first. When a second standard for insulin was required, consisting of the pure, crystalline hormone, this was prepared in the home

INTERNATIONAL BIOLOGICAL STANDARDS

No.	Standard preparation	Adopted in	International unit milligrams	International distributing centre
1	Diphtheria Antitoxin	1922	0·0628	State Serum Institute, Copenhagen.
2	Tetanus Antitoxin	1928	0·1547	
3	Antidysentery Serum (Shiga)	1928	0·0500	
4	Antipneumococcus Serum (Type I)	1934	0·0886	
5	,, ,, (Type II)	1934	0·0894	
6	Staphylococcus α-Antitoxin	1934	0·500	
7	Gas Gangrene Antitoxin (perfringens)	1931	0·2660	
8	,, ,, ,, (Vibrion Septique)	1934	0·2377	
9	,, ,, ,, (oedematiens)	1934	0·2681	
10	,, ,, ,, (histolyticus)	1935	0·3575	
11	Old Tuberculin	1931	—	
12	Diphtheria Antitoxin for Flocculation Test	1935	—	
13	Vitamin A (mixed carotenes)	1931	0·001	National Institute for Medical Research, Hampstead, London.
	(pure β-carotene)	1934	0·0006	
14	,, B_1 (adsorption product of vitamin B_1)	1931	10·0	
	(pure synthetic vitamin B_1)	1938	0·003	
15	,, C (*l*-ascorbic acid)	1934	0·05	
16	,, D (irradiated ergosterol solution)	1931	1·0	
	(calciferol)	1934	0·000025	
17	Arsphenamine	1925	—	
18	Neoarsphenamine	1925	—	
19	Sulpharsphenamine	1925	—	
20	Insulin (dry insulin hydrochloride)	1925	0·125	
	(pure crystalline insulin)	1935	0·0455	
21	Pituitary (posterior lobe) powder	1925	0·5	
22	Digitalis	1925	80·0	
23	Ouabain	1928	—	
	Oestrus-producing hormones:			
24	(i) -oestrone	1932	0·0001	
25	(ii) -oestradiol monobenzoate	1935	0·0001	
26	Androsterone (for male hormone)	1935	0·1	
27	Corpus Luteum Hormone (progesterone)	1935	1·0	
28	Chorionic Gonadotrophin	1938	0·1	
29	Serum Gonadotrophin	1938	—	
30	Thyrotrophin	1938	—	
31	Prolactin, Galactin or Mammotrophin	1938	—	

of insulin, Toronto, and a suitable portion was allotted for distribution in the Old World, the American continents drawing their supplies from Toronto. When further national standards were required by the British Pharmacopoeia of 1932, we had those for digitalis and strophanthus ready, as well as those for the vitamins,

which we had prepared and were holding also on the international account. The position will be the same when standards for the sex hormones and the anterior pituitary principles become official in this country; for in all these instances the standards have been prepared by us on behalf of the International Organisation, and portions set aside to constitute the official standards for this country. The National Institute, accordingly, has accepted, and is discharging, a triple obligation in relation to these standards—on behalf of the International Health Organisation under the League of Nations, of the Administration of the Therapeutic Substances Act and of the British Pharmacopoeia; although we arrange for these functions to overlap so far as is proper and convenient, they are not coterminous. The International Standards are distributed all over the world, in some instances to as many as 33 countries. Wherever possible we send them to National Control Centres, charged like our own with the duty of making equivalent national standards, or, for some standards, with the further distribution, in their respective countries, of supplies of the international standard material furnished for the purpose.

You might suppose that this work would involve a large department, with a numerous staff giving whole time to the work. It has not done so. My colleague, Dr. Hartley, who acts as Director of all our Standards work, has indeed given a large part of his time to it over the past 17 years, devising the methods by which the standard preparations are prepared and stabilised in our own and many other laboratories, and making himself responsible with the administrative staff of the Institute, in particular with Dr. Chalmers, now our Medical Administrative Officer, for the complex organisation which the growth of the work has entailed. Much of the investigatory work has been undertaken by our colleagues on the regular Research Staff of the Institute, in our sections of Bacteriology, Pharmacology and Endocrinology, who have accepted various items, as they have arisen, as parts of their research programmes. A theoretical case could, I think, have been made for a separate department charged with these important duties, and separately financed, instead of distributing the work through the Institute, supported from the general research funds of the Medical Research Council, apart from a small annual contribution from the League of Nations. In practice, however, I am inclined to think that we adopted the better plan; for I believe that, if work of this kind is to be done with the right spirit of enterprise and initiative, it is often done best by those engaged also in wider fields of research; and I believe, also, that many men engaged in more fundamental researches into the unknown can strengthen their equipment, by some degree of contact with work directed to providing the means of quantitative precision, consistency and uniformity, in the practical application of what is known already.

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The Ultra-Violet Absorption of Calciferol

By S. K. CREWS, Ph.C., A.I.C., and E. LESTER SMITH, D.Sc., F.I.C.

Published figures for the ultra-violet absorption ($E^{1\%}_{1\,cm.}$ $265m\mu$) of calciferol (vitamin D_2) vary from 495 to 460.[1,2] Suggested standards have been put forward[3,4] including those of the Monograph in the 1936 Addendum to the B.P. 1932.

It was found that the spectrophotometric determinations of calciferol carried out in the analytical departments of The British Drug Houses, Ltd., and Glaxo Laboratories, Ltd., were not in agreement, although determinations made on solutions of potassium nitrate and potassium chromate did agree; a collaborative investigation of these discrepancies was therefore undertaken by members of the scientific staffs. For this purpose operators exchanged plates and samples and visited each others laboratories to compare techniques.

Photometers.—Of the three instruments used in this investigation, two were fitted with Spekker photometers and the third with a modern short-focus rotating sector photometer. In all the experiments the light source used was a condensed spark between tungsten steel electrodes.

Table I

Instrument	Calciferol "A" $E^{1\%}_{1\,cm.}$ $265m\mu$	Calciferol "B" $E^{1\%}_{1\,cm.}$ $265m\mu$
Sector photometer ..	412·5	421
Spekker photometer ..	430*	475

* 0·0416 per cent. solution in 1-mm. cell. Same solution diluted 1 : 20 in 1-cm. cell gave a value of 445. Total times of exposure 43 seconds and 5 seconds, respectively.

It was shown that the "spotting" in the two laboratories of the same spectra on the plates led to no significant differences in the values deduced therefrom. Different samples of calciferol all gave appreciably lower values for $E^{1\%}_{1\,m.}$ $265m\mu$ when examined with the rotating sector instrument than with the others (see Table I). The time of exposure of the calciferol to radiation was very much longer in this instrument than in those equipped with Spekker photometers, and it soon became apparent that shorter exposures led to higher values. If the same calciferol solution was used for successive determinations and was thus subjected to increasingly prolonged irradiation, decreasing values were obtained; this result was obtained in both laboratories. (See Tables II and III.) It was also established that the rate of photo-chemical change was about the same in isopropyl alcohol as in cyclohexane solution (see Table IV).

Table II

Instrument	Total time of exposure Seconds	Calciferol "A" 0·0022 per cent. in isopropyl alcohol in 1-cm. cell $E^{1\%}_{1\,cm.}$ $265m\mu$	Per cent. of initial E	Calciferol "B" 0·002 per cent. in isopropyl alcohol in 1-cm. cell $E^{1\%}_{1\,cm.}$ $265m\mu$	Per cent. of initial E
Sector photometer	34	469	100	483	100
	79	404	86	446	92
	106	375	80	411	85
	129	346	74	371	77

TABLE III

Instrument	Calciferol "C" 0·0406 per cent. in ethyl alcohol in 1-mm. cell: Total time of exposure Seconds	$E^{1\%}_{1\,cm.}$ 265mμ	Per cent. of initial E	Calciferol "B" 0·0409 per cent. in ethyl alcohol in 1-mm. cell: Total time of exposure Seconds	$E^{1\%}_{1\,cm.}$ 265mμ	Per cent. of initial E
Spekker photometer (1)	32	470	100	18	465	100
	101	425	90	85	440	95
	133	410	87	164	405	87
	182	395	84	225	380	82
	230	370	79	286	370	80
	281	345	73	—	—	—
Spekker photometer (2)	3	503	100	—	—	—
	33	487	96·8	—	—	—
	108	437	86·9	—	—	—
	210	412	81·9	—	—	—

TABLE IV

Instrument	Calciferol "D": Total time of exposure Seconds	0·002 per cent. in isopropyl alcohol in 1-cm. cell: $E^{1\%}_{1\,cm.}$ 265mμ	Per cent. of initial E	0·002 per cent. in cyclohexane in 1-cm. cell: $E^{1\%}_{1\,cm.}$ 265mμ	Per cent. of initial E
Sector photometer	38	491	100	507	100
	69	447	91	458	90·3
	98	421	85·7	435	85·8
	125	397	80·8	410	80·8
	157	361	73·5	373	73·5

TABLE V

Instrument	Batch No.	Normal technique $E^{1\%}_{1\,cm.}$ 265mμ	Continuous flow method $E^{1\%}_{1\,cm.}$ 265mμ
Spekker photometer (1)	"E"	460	485
	"F"	475	485
	"G"	475	490
	"H"	480	490
	"I"	475	490
	"J"	480	490
	"K"	480	500
Spekker photometer (2)	"L"	476 (2 secs.)	—
	"M"	495 (3 ,,)	—
	"N"	503 (3 ,,)	—
	"O"	495 (2 ,,)	495 (2 secs.) 12 ml. per min.
		446 (102 ,,)	495 (2 ,,) 104 ,, ,, ,,
	"P"	480 (2 ,,)	—
	"Q"	478 (2 ,,)	—
	"R"	488 (2 ,,)	—

Determinations were also made in a special cell, arranged so that the solution flowed through the cell during the spectrophotometric determination. In this way slightly higher values were obtained than by the normal technique with the

shortest possible exposures and the smallest number of exposures necessary to determine the absorption at the maximum of the curve (see Table V).

It seems that the extreme photo-lability of calciferol necessitates special precautions for the accurate determination of its absorption spectrum, even when the most suitable instrument is used for the purpose. It should, moreover, be realised that the risk of error from this source is frequently present in determinations of absorption spectra in the visible region of the spectrum (including visual colorimetry) as well as in the ultra-violet.

In our opinion the continuous-flow method offers the most accurate means of determining the extinction coefficient of calciferol and other similar substances whose absorption spectrum is affected by exposure to ultra-violet radiation.

Our thanks are due to Mr. J. H. Singer and Miss B. E. Stern for assistance with the spectrophotometric determinations, and to the Directors of Glaxo Laboratories, Ltd., and The British Drug Houses, Ltd., in whose laboratories this work was carried out, for permission to publish these results.

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Notes on the Examination of Textiles in Cases of Suspected Dermatitis

BY H. E. COX, D.Sc., PH.D., F.I.C.

(*Read at the Meeting, May* 5, 1939)

ALL those who have had experience of cases of dermatitis alleged to arise from contact with articles of clothing or materials which are worn next to the skin, will agree that they often present much difficulty. It is now fairly well established that certain chemicals are irritant and when these are present the chemist feels himself on sure ground with regard to his part in the matter. But it happens in a great number of cases that no substance that could ordinarily be regarded as irritant is present or that substances which are present are only in such quantity as is normal to the particular garment. So the analyst may have not only to determine what is present, if anything, but he must be able to give some indication as to the importance or quantitative significance of what he finds. Both these problems are often really difficult.

The legal aspects of claims have been somewhat clarified by certain recent judgments of the courts. These have set out more distinctly the relations between

buyer and seller or manufacturer; but they have also, in my opinion, brought more clearly into relief the scientific difficulties—both medical and chemical—and they throw on the experts the onus of proving or disproving certain things which formerly did not appear to arise.

I will refer briefly to two or three cases of fundamental importance. The principles that these cases establish are fairly obvious once they are set out in the clear terms which our judges usually employ. In *Grant* v. *The Australian Knitting Mills*, the underpants complained of contained about 1500 p.p.m. of sulphur dioxide, and the Privy Council upheld the view that the disease contracted and damage suffered were caused by this sulphur dioxide, which connoted a defective condition of the garment. The plaintiff's skin was held to be normal. It is now well known, as shown by Trotman and Bramley[1] that stored wool may often contain up to 0·25 per cent. or more of sulphur dioxide, and there is no doubt that millions of garments are worn daily which contain such quantities of sulphite. So if the plaintiff's skin was normal might it not equally have been urged that so were the pants! In *Griffiths* v. *Peter Conway*[2] the result was otherwise; the garment was normal, but the plaintiff was not, and the Court of Appeal emphasised the point that the essential matter for the seller to know in such cases with regard to the purposes for which the article is required consists in the particular abnormality or idiosyncrasy from which the buyer suffers. The fact that these essential characteristics are not known by the buyer is immaterial. So it is apparent that when dermatitis follows the wearing of a garment and there is no clear cause for it, the question whether the sufferer is abnormal or the garment abnormal becomes paramount. The chemist can only deal with the garment, and even when dealing with this encounters great difficulties. Moreover, in cases of doubt the court will often have regard to the fact that the plaintiff's state was induced by something. Thus, in a recent case in which a very unlikely substance was suggested but strongly denied by the defence, the learned Judge said: "Unless I can find in the case some plain evidence which satisfies me that there is some cause of the state of the plaintiff other than some irritant substance in the suit of clothes . . . I am driven to the conclusion that *something* of that sort must have been there." It cannot be easy to determine whether or not an individual is normal towards a number of chemical substances or natural products; but it ought to be within the competence of the chemist to determine whether a garment is normal or not, and it is to this aspect that I wish to draw attention. What substances should be expected in various garments and what quantities of substances which are common in them? Further, what impurities or possibly nocuous compounds may be anticipated as being possible. The latter might seem to be superfluous, but in my experience it is not so, for one finds at times allegations of the presence of compounds which have not been used at all in the materials concerned, and could not ordinarily arise. Hence it may be useful to record a few points gathered from a rather extensive experience.

Acidity.—It is often alleged that the suspect garment was acid. Garments usually are; but how much and what acid is present, and is it excessive? Cotton fabrics, in my observation, usually show a faintly acid reaction (pH $< 6{\cdot}0$) to litmus or lacmoid but not to methyl red; titration of the acid shows about 0·1 per

cent. reckoned as sulphuric acid. Wool presents some difficulty. Undyed bleached wool is always acid to a relatively high degree, an apparent acidity of 2 or even 2·5 per cent. being common; dyed woollen cloth is generally less acid, and in my observation seldom contains more than 1·5 per cent., reckoned as sulphuric acid, and about 0·7 or 0·8 per cent. is usual. Methods for the determination of acids in wool have been critically examined by Barritt *et al.*,[3] and study of their paper shows the difficulties of the subject. In my view the distillation method is most convenient. It is important, too, to determine whether acetic or formic acid is present; this also may be done by distillation. A further point to be borne in mind is that the acidity of woollens commonly increases with age and exposure, and is influenced by the dyestuffs present. There is evidence (*cf.* P. R. McMahon and J. B. Speakman[4]) that the disulphide linkages are hydrolysed in light to sulphide and this may be oxidised to sulphite and sulphate. Again, with acid dyestuffs the colour is usually more fast to water when the *p*H is low; if the wool has been dyed from an acid bath a greater amount of the dye actually enters into combination with the fibres and less base is available to mineral acid.

Most rayons and union fabrics are slightly acid, but the acidity, on distillation from these materials, is usually less than 1 per cent.

In hat leathers acidity may be of much importance; it should be determined by the standard Proctor–Searle method, and when so determined should not exceed about 0·5 per cent. Direct titration methods give quite misleading results, and the Proctor–Searle method is not applicable to chrome or semi-chrome tanned leather; for these, Innes's procedure is available. Some idea of the nature of the acid may be gained by the Innes method,[5] which consists in observing the *p*H value of a 2 per cent. aqueous extract before and after dilution with 10 volumes of distilled water. If a mineral or strong acid is present the difference figure on dilution will be greater than 0·7. The presence of chromic acid or formic acid should also be investigated.

SULPHITE.—In view of the Australian Knitting Mills case, to which I have referred, sulphur dioxide is important. It is not usually present in cotton or rayon fabrics, but is common in wool—so much so that one may be permitted to doubt whether it can truly be regarded as an irritant to normal persons. The sulphite in woollen clothing is not all removed by washing or extraction with hot water. Quite a considerable quantity will survive two or more launderings. As complex sulphides and some hydrogen sulphide may be formed in wool by the action of light, it is essential to accuracy to use hydrogen peroxide (not iodine or bromine) for the oxidation when determining the sulphite by steam-distillation after acidifying with a few drops of phosphoric acid. If no phosphoric acid is added the natural acidity of the wool does not suffice to liberate all the sulphite; evidence can be adduced to show that the sulphite is in part combined with the wool. Trotman reports 0·25 per cent. as fairly common and 0·5 per cent. as occasional; in my experience 0·15 to 0·2 per cent. is more usual in bleached garments and in dyed woollens one usually finds none, or only an insignificant trace.

CHROMIUM.—On account of the well-known lesions produced in chrome workers the presence and significance of chromium in a case of alleged dermatitis requires careful consideration. Here, again, we are considering an element which

is very widely used and is to be found in millions of garments. It arises in three principal ways: chrome tanning in leather such as shoes, gloves or jackets; as an after-chroming process in fixing or brightening the colours in hat leathers for example; as a mordant in the application of vat and other dyes to textiles. Whether any chromium is present at all is easily determinable; if it is present it becomes important to know (*a*) its state, anionic or cationic, and (*b*) how much, if any, is soluble; also what solvent should be used and at what temperature.

Chromates are undoubtedly more likely to be irritant than cationic chromium in the forms of salts. The former, even in minutest amounts, gives an immediate reaction with diphenyl carbazide, whereas small concentrations of chromium salts do not so react. When chrome mordants are employed in dyeing, the amount used may be about 2 per cent. as dichromate reckoned on the material. After dyeing and washing the whole of this is, in my observation, combined with the fabric and the dye; none is extracted either by water or weak acid solutions at blood heat. In such case it can hardly be supposed to have any physiological activity. Such combined chromium may also be found in silk and some union fabrics containing rayon. Chrome-tanned leathers, such as shoe linings, may contain quite large quantities of total chromium and hat leathers may have small quantities. It is most unusual to find any soluble chromate in such products, but common to find small traces, such as 0·1 per cent., of soluble chromium (expressed as CrO_3) extractable by hot water and a like quantity extractable by weak acid (say 1·0 per cent. acetic acid) at blood heat. If these are the normal occurrence, at what stage is it to be thought that irritant properties arise or that the garment is abnormal? In my view the proper or best extraction media are (*a*) water and (*b*) 1 per cent. acetic acid, or the Society of Dyers and Colourists' acid artificial perspiration liquor,[6] extracting at blood heat.

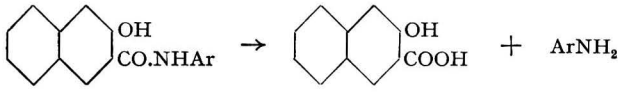
Metals may be present as necessary mordants or as co-ordination compounds in certain complex dyes, or as a result of after-treatment of the material. Amongst the metals which may be met are antimony, lead, tin, zinc, copper, titanium, nickel and manganese; it is not suggested that these can be considered as irritant, but they need to be determined, if present, and their solubility taken into account. Iron is almost omnipresent as a natural constituent, and aluminium is common in waterproofed garments; most of it is insoluble in water, but much may be extracted with acid. I know of no reason to think that it is harmful. The amount extractable in hot water may be about 0·1 per cent. as aluminium.

In the practical investigation of a specimen it is generally convenient first to determine the acidity, sulphite, mineral matter and any other inorganic constituents and then to proceed to the study of the organic constituents including dyes and intermediates, if any. In considering the organic compounds which may be concerned, having regard to the complexity and variety of modern dyes, I venture to emphasise the importance of the preliminary study of the material and its dyes. Direct application of special tests for individual intermediates is most liable to error and causes subsequent disputes. First, one must observe the nature of the material; this is not always easy with certain rayons. If it is a composite fabric the constituent parts may be examined separately. Then, if there is a pattern, it is well to note whether it results from printing-on with a dye

or from weaving of dyed threads; these points are elementary, but they do help in arriving at the truth, for it is only when one has ascertained the nature or grouping of the dyes present that a reliable scheme of testing can be arranged. For example, a cloth dyed with indigo will not contain a diamine, and an acetate rayon will not be in the least likely to contain an amino-phenol. Realisation of such facts would avoid disputes such as have at times arisen. It may be necessary to test for these unlikely substances and if so the interfering dye must first be eliminated. When two or more kinds or colours of fibre are present it is best to examine them separately. In addition to the well-known textbook of A. G. Green, tables prepared by Clayton[7] and by Keyworth[8] are most useful. It is usually impossible to identify all the dyes present, but one should at least ascertain their class or type. Then reference to standard works, such as the Colour Index and Prof. Rowe's recent lectures to the Institute of Chemistry, will give an indication of what intermediates are likely to have been used and what decomposition products of azo-dyes, for example, are possible. A dye formed from, say, benzidine and an amino-naphthol-sulphonic acid will not contain *p*-phenylenediamine.

Next comes an examination of the intermediates, if any are present; they may generally be extracted from the fabric by organic solvents, and some work by Forster and Hanson[9] is very helpful in this direction (*cf.* also Rowe and Levin[10]).

Certain types of rayon are commonly dyed either with amino-anthraquinone derivatives, which appear to dissolve in cellulose esters, or with azoic colours which are based upon aryl amides of β-hydroxy-naphthoic acid, such as naphthol A.S. I have found that free hydroxy-naphthoic acid is sometimes present, possibly as a result of hydrolysis of the amides, and it seems that it is a potential irritant to some people. So far as is known, the anilides or other amides are harmless. These amides are hydrolysed by boiling with acid or alkali, thus



Hence, if an azoic colour is found, it is important to consider what products of hydrolysis, if any, are present; to do this, a quantity of the amide must be hydrolysed and the products separated; it is doubtful whether a certain diagnosis can be made without such work. Many acetate rayons contain small amounts of naphthol A.S. or allied compounds; the quantity may be as much as about 0·2 per cent., though it is usually less. Cotton fabrics may also contain insoluble azo-colours or pigments; these may be separated by dissolving a piece of the material in dilute sulphuric acid and re-crystallising the dye as described by Rowe and Levin,[10] but such procedure should only be used to identify the dye and determine its decomposition products, for it is obvious that it will involve some hydrolysis of the dyestuff. When it has been ascertained what substances are liable to be present it is not difficult to extract any of the free or uncoupled intermediates. Only in such ways is it possible really to determine whether a fabric is normal or whether it contains a supposed irritant.

Another point that is worthy of consideration is the fastness, or otherwise, of the dye in a fabric. There is an increasing number of insoluble vat colours which

are dissolved as either leuco compounds or salt complexes of esters and applied so as to form an insoluble dye on both animal and vegetable fibres. If a dye is completely fast and insoluble, it is difficult to see how it can affect the skin. But what if much dye bleeds into water or artificial sweat mixtures? It does not seem reasonable to suppose that a dye is irritant just because it is soluble or will stain the skin. Lipsticks and cosmetics generally contain such colours. Yet one sometimes sees solubility made the basis of some alleged injury. Thus it is important to record the facts, and for this purpose the procedure and reagents proposed by the Society of Dyers and Colourists[6] should be used.

At times certain waterproofed garments have come under suspicion; it may therefore be pointed out that, in addition to aluminium soaps and such waterproofing materials, they are likely to contain mineral pigments such as khaki or iron buffs instead of the familiar synthetic colours. Most garments treated with aluminium soaps or wax compounds yield small quantities of soluble aluminium on extraction, but there is no reason to suppose that such aluminium is irritant. In general, I see no reason why a garment should be alleged to contain an irritant unless it contains something definitely known as such. But it is desirable to obtain and collect reliable data as to what substances and quantities are present; such accumulated information should be valuable in the interests of justice between manufacturers and their customers.

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Electrolytic Determination and Separation of Bismuth

Part II. Determinations in Sulphuric and Nitric Acid Solutions

By F. G. KNY-JONES, M.Sc.

Methods of determining bismuth by electro-analysis in sulphuric acid solution hitherto described have been slow and applicable only to small amounts of the metal.

Hollard and Bertiaux,[1] using a method based on an earlier one of Smith and Knerr,[2] obtained good results for amounts of bismuth not exceeding 0·1 g. in dilute acid solutions, with a current of 0·1 amp. for 48 hours. Scott and Mulligan[3] describe a method for amounts of less than 0·03 g.

Brunck,[4] whose work is quoted in standard analytical textbooks,[5] does not advise the use of sulphuric acid solutions, on the ground that excessive amounts of acid must be added to prevent the formation of basic salts.

Sand,[6] using controlled potential, obtained good deposits of antimony from strongly acid solutions at high temperatures, and was able to effect a rapid separation from tin by this method. By the use of similar conditions for bismuth it has now been found possible to make a rapid determination of that metal in amounts large enough for ordinary analytical work. It should, perhaps, be mentioned that the reason for using controlled potential for this determination is that bismuth generally forms spongy deposits if the electrode potential is not kept as low as possible.[7]

Experimental

Determination in Sulphuric Acid Solution.—Samples of pure bismuth were weighed out and dissolved by heating with 10 ml. of conc. sulphuric acid; in some of the experiments 1 ml. of nitric acid was added to effect more rapid solution of the metal. The solution was cooled and diluted with water, and its volume was adjusted to contain from 20 to 25 ml. of sulphuric acid per 100 ml. One g. of hydrazine sulphate was added as depolariser, and the solution was heated to about 100° C. The electrolysis was carried out at this temperature, with the use of the platinum gauze electrodes, the auxiliary electrode and voltmeter described by Lindsey and Sand.[8]

A saturated calomel auxiliary electrode with a potassium chloride connection was used, the initial potential being 0·055 volt. When the current fell to zero, the auxiliary potential was raised in two or three steps to 0·15 volt and the electrolysis ended when the current fell to zero under this voltage. The deposited metal was washed and dried in the usual manner. Typical results are given in the following table:

Expt. No.	Initial current in amp.	Bismuth taken g.	Bismuth found g.	Temp. °C.	Time Minutes
1	1·0	0·1323	0·1327	100	15
2	1·2	0·2536	0·2537	105–106	25
3	0·4	0·2009	0·2007	90–105	17
4	0·7	0·1525	0·1525	96–104	10
5	0·4	0·2003	0·1996	95–102	30
6	1·0	0·1298	0·1294	95–100	15
7	0·4	0·1796	0·1795	95–99	20

The deposits obtained were of good appearance and adhered well to the electrode.

Determination in Nitric Acid Solution.—In the course of this work some depositions have been made from nitric acid solutions under the conditions described by Sand[9] and Collin,[10] and the following notes were made:

(1) Small amounts of chlorides (*e.g.* 0·05 g. of potassium chloride) cause precipitation of bismuth oxychloride, and poor, non-adherent deposits of the metal result. By increasing the amount of conc. nitric acid present it is possible to obtain satisfactory deposits, but the presence of chlorides should be avoided.

(2) The deposition can be carried out successfully in the presence of sulphates; hence in the absence of lead, hydrazine sulphate may be used as depolariser instead of the less stable hydrate.

(3) Experiments were made in which the platinum wire described by Brown[11] was used as auxiliary electrode. The method was similar to that described in Part I of this work[12] and, with a P.D. (ΔE) between the cathode and the wire electrode of 0·03 volt rising to 0·1 volt at the end of the electrolysis, good deposits having the correct weight were obtained.

I wish to express my thanks to Dr. H. J. S. Sand for his help and interest.

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March, 1939

Determination of Aluminium by Precipitation with 8-Hydroxy-Quinoline from Mineral Acid Solution; Separation from Large Amounts of Magnesium

BY G. STANLEY SMITH, B.Sc., A.I.C.

THE most rapid method of determining aluminium is undoubtedly by way of its crystalline 8-hydroxyquinoline complex,[1,2] $Al(C_9H_6ON)_3$, which is precipitated within the *p*H range 2·8 to 12·3, the limits for quantitative precipitation being 4·2 and 9·8.[4] The compound is easy to filter off and is practically insoluble in boiling water. It is, however, readily soluble in conc. hydrochloric acid and the solution, after dilution, can be titrated with bromate-bromide solution.

$$C_9H_7ON + 2Br_2 = C_9H_5ONBr_2 + 2HBr.$$

For precipitation in acid solution the published methods[2] describe the addition of an acetic acid solution of the reagent to the warm, slightly acid, aluminium solution previously or afterwards treated with excess of ammonium or sodium acetate. In the present paper another method of obtaining a precipitate is described

and put forward as a possible improvement on the older methods, which can yield erratic results. The procedure involves a minimum of manipulation, the results are quantitative, and a sharp separation from a large amount of magnesium is achieved in one operation.

Since magnesium forms a complex which begins to be precipitated if the *p*H rises to slightly over 7, the complete precipitation of aluminium free from magnesium obviously requires close control over the acidity of the solution. After trials of various reagents for automatic control of the acidity the possibility of using a mixture of bromate and bromide, in conjunction with a reagent for removing bromine as soon as it is formed, was investigated.

A bromate-bromide solution was used by Gooch and Osborne[5] in their experiments on the hydrolysis of metallic salts. They obtained practically complete hydrolysis of aluminium sulphate when its aqueous solution was boiled for a long time with bromate-bromide. More recently Moser and Iranyi[6] by similar means reduced the acidity of a hydrochloric acid solution from about 0·1 *N* to 0·002 *N*.

Sodium thiosulphate is stated[7] to react with bromine according to the equation:

$$Na_2S_2O_3 + 8Br + 5H_2O = Na_2SO_4 + 8HBr + H_2SO_4$$

If this equation is considered in relation to that for the reaction between bromate, bromide and acid:

$$5KBr + KBrO_3 + 6HCl = 6KCl + 3H_2O + 6Br,$$

it appears that, in the presence of sufficient bromide, the addition of bromate and thiosulphate to an acid solution in the molecular proportions 4 : 3 should increase the acidity—an effect the opposite of that desired. However, the behaviour of a solution containing a large excess of thiosulphate pointed the way to achieve the end in view. This solution contained, in 500 ml., 60 g. of potassium bromide, 14 g. of potassium bromate and 125 g. of sodium thiosulphate. Tests were carried out as follows:

(1) Ten ml. added to 100 ml. of cold water gave a solution alkaline to methyl red and acid to phenol red, requiring only one drop of 0·1 *N* acid or alkali to change the colours of the indicators. On boiling, the solution became alkaline to phenol red.

(2) Ten ml. added to 100 ml. of boiling water containing 2 ml. of *N* hydrochloric acid remained acid to phenol red on boiling for one minute. On cooling and adding one drop of 0·1 *N* sodium hydroxide, the solution became red (alkaline), but the yellow colour returned on boiling, though the solution remained alkaline to methyl red during a further 15 minutes' boiling, which concluded with the appearance of a cloudiness due to the separation of sulphur.

(3) Ten ml. added to 100 ml. of boiling water containing 7 ml. of *N* hydrochloric acid and boiled for one minute gave a pure yellow colour with methyl red (alkaline), but, on cooling, a pink tinge appeared. Further boiling caused a development of the red colour and, after 5 minutes, the solution became cloudy.

(4) With 6·5 ml. of *N* hydrochloric acid present initially, the solution had to be boiled 3 minutes to give an acid reaction to methyl red.

(5) With 5 ml. of *N* hydrochloric acid the boiling solution was alkaline to methyl red after one minute's boiling, as also after a further 5 minutes.

These results indicate that a solution containing between 2 and 7 ml. of *N* hydrochloric acid and 10 ml. of the bromate-bromide-thiosulphate solution in 110 ml. will, after boiling for one minute, react alkaline to methyl red and acid to phenol red and, according to the table given by Kolthoff,[8] have a *p*H between 6·0 and 7·0.

METHOD.—The following method is suitable for the determination of amounts of aluminium that can conveniently be titrated with 0·1 *N* bromate-bromide solution:

Reagents.—8-Hydroxyquinoline, 3 per cent. solution in 0·2 *N* hydrochloric acid; ammonia solution, dilute, approximately 2 *N*; hydrochloric acid, dilute, approximately *N*; bromate-bromide-thiosulphate "buffer," as above; bromate-bromide standard solution, 0·1 *N* (see also below); thiosulphate solution, 0·1 *N*; 10 per cent. potassium iodide solution; starch solution.

Procedure.—To the slightly acid solution (about 100 ml. in volume, containing up to 0·01 g. of aluminium) add 10 ml. of the hydroxyquinoline reagent, heat to boiling and add dilute ammonia to the well-stirred solution until one drop produces a cloudiness that remains. Add 3 ml. of *N* hydrochloric acid and boil for a few seconds to give a clear solution. Then add, all at once, to the well-stirred solution 10 ml. of the acidity-regulating solution and boil gently for one minute after boiling begins again. Filter at once through a paper-pulp pad and wash several times with nearly boiling water. Remove the excess of water from the funnel by suction, transfer the pad with forceps to a 500-ml. conical flask, wipe the funnel with a piece of moist filter-paper and put the paper into the flask. Add 30 ml. of conc. hydrochloric acid, shake the flask with a rotatory motion and leave it on a boiling water-bath for two or three minutes to ensure solution of the precipitate. Add 90 ml. of cold water, mix and cool in running water, add a slight excess of the bromate titrating solution, then 10 ml. of 10 per cent. potassium iodide solution, and titrate the iodine liberated by the excess of bromate with thiosulphate (see below).

$$1 \text{ ml. } 0{\cdot}1\ N \text{ bromate} \equiv 0{\cdot}0002248 \text{ g. aluminium.}$$

For amounts of aluminium less than 5 mg. the presence of 5 to 10 g. of ammonium chloride is to be recommended, and the process is slightly modified as follows:—To the solution containing ammonium chloride add a few drops of methyl red indicator (non-alcoholic) and then dilute ammonia carefully until the indicator changes colour. This is followed by 10 ml. of the hydroxyquinoline reagent, 1 to 2 ml. of *N* hydrochloric acid and, after boiling to produce a clear solution, 10 ml. of the "buffer" solution.

Titration.—An accurate direct titration with bromate is impossible, but methyl red or indigo-carmine can be used as internal indicator to show when an excess of bromate has been added. Since the indicator is gradually oxidised during the addition of bromate, it is usually necessary to introduce from time to time further quantities of the indicator. This is troublesome, and for accurate work a correction has to be applied.

I have found that the indicator (indigo-carmine) may, with advantage, be dissolved in the bromate solution. The correction, once determined, is then valid for all future titrations, as it is exactly proportional to the volume of bromate

used and appears in the calculation only as a constant factor for the solution. A suitable quantity is 25 ml. of 0·5 per cent. aqueous indigo-carmine in 1 litre of 0·1 *N* bromate-bromide solution, the factor of which will be reduced by about 0·75 per cent. A solution containing in 1 litre 2·805 g. of potassium bromate, 12 g. of potassium bromide and 25 ml. of 0·5 per cent. indigo-carmine solution will probably be exactly 0·1 *N*. If the amount of potassium bromide is doubled, much of the indicator is thrown out of solution on standing. It should be mentioned that the bromate solution containing indigo-carmine must be used for titrating cold solutions only.

During a titration with this solution the hydroxyquinoline solution soon becomes deep green, and it remains green until, in the neighbourhood of the end-point, it tends to yellowish-green. If the bromate is then added slowly to the well-agitated solution, there appears, 1 to 3 ml. past the calculated end-point, a yellow colour nearly free from green. Potassium iodide may then be added at once and the iodine titrated with thiosulphate, starch solution being used. The end-point is very sharp, the final colour being a greenish-yellow.

The use of carbon disulphide[9] before the thiosulphate titration, besides being unpleasant, is quite unnecessary,[3] and may very well be abandoned. If the acidity after the addition of bromate is 2 to 3 *N*, no trouble is likely to arise.

The results shown in the table were obtained by the method described above. The aluminium taken was in the form of a solution of pure aluminium in hydrochloric acid or of aluminium ammonium sulphate. In each test the volume before addition of 10 ml. of the buffer solution was 100 to 110 ml., and the solution contained 10 ml. of 3 per cent. hydroxyquinoline together with any ammonium chloride or magnesium, as chloride or sulphate, in the amounts shown.

Aluminium mg.	Ammonium chloride g.	Magnesium g.	Titration, 0·1 *N* bromate required ml.	Calculated titre ml.
10·00	—	—	44·5; 44·6	44·5
10·00	6	0·3	44·6; 44·65	44·5
7·50	—	—	33·4; 33·5; 33·5	33·4
7·50	6	—	33·45	33·4
7·50	6	0·8	33·6	33·4
7·50	6 (also 1 g. of tartaric acid)	0·8	33·4	33·4
7·50	do.	—	33·45	33·4
5·00	—	—	22·25; 22·25	22·25
5·00	—	1·0	22·25; 22·25	22·25
5·00	—	5·0	22·5	22·25
2·50	—	—	10·6; 10·9	11·1
2·50	10	—	11·05; 11·1	11·1
1·25	—	—	5·0; 5·1	5·55
1·25	10	—	5·5	5·55
0·50	10	—	2·15	2·2

The solution employed for regulating the acidity in the precipitation of aluminium with hydroxyquinoline should also be applicable to the precipitation of

aluminium hydroxide in presence of manganese and magnesium. The *p*H for the precipitation of the hydroxides of the latter metals could never be attained, even locally.

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April 28th, 1939

First Report of the Poisons Sub-Committee appointed to investigate Methods of Assay for Various Substances appearing in the Poisons Schedules of the Poisons Regulations, 1935, to the Analytical Methods Committee of the Society of Public Analysts and other Analytical Chemists

I. ASSAY OF LOBELIA (*LOBELIA INFLATA*)

MEMBERS OF THE SUB-COMMITTEE.—G. Roche Lynch, O.B.E., M.B., B.S., F.I.C. (*Chairman*); N. L. Allport, F.I.C.; C. E. Corfield, B.Sc., F.I.C.; C. Edwards, B.Sc., F.I.C.; D. C. Garratt, B.Sc., Ph.D., F.I.C.; C. H. Hampshire, M.B., B.S., B.Sc., F.I.C.; W. H. Linnell, M.Sc., Ph.D., F.I.C.; W. A. N. Markwell, A.I.C.; J. R. Nicholls, B.Sc., F.I.C.; A. D. Powell, F.I.C.; A. I. Robinson, Ph.C.; C. E. Sage, F.I.C.; Norman Evers, B.Sc., F.I.C. (*Hon. Secretary*).

SURVEY OF METHODS.—The most important methods previously published for the assay of lobelia are as follows:

Vanderkleed and E'We (*J. Amer. Pharm. Assoc.*, 1916, **5**, 713).
Wieland (*Ber.*, 1921, **54**, 1784).
Mascre (*Bull. Sci. Pharm.*, 1930, **37**, 209).
Peyer and Gstirner (*Arch. Pharm.*, 1932, **270**, 44).
Markwell (*Pharm. J.*, 1936, **136**, 617).

The methods of Vanderkleed and E'We and of Mascre were tried and compared with that of Markwell. The last method was found to be the most satisfactory in working and to give the most uniform results. The Sub-committee decided

to concentrate on Markwell's method, and a sample was circulated on which the method as published was carried out. The results were as follows:

Member	Alkaloids as lobeline: by titration Per Cent.	Alkaloids as lobeline: by weighing Per Cent.
A	0·51	—
	0·52	—
	0·51	—
B	0·43	—
C	0·51	0·62
	0·49	0·63
	0·51	0·57
D	0·45	—
	0·46	—
E	0·44	—
	0·43	—

The results of three of the workers were clearly lower than those of the other two. It was suggested that the quantity of ammonia added to precipitate the alkaloid might have some effect on the result. Comparative tests were therefore carried out (*a*) adding a slight excess of ammonia, and (*b*) adding 5 ml. of dilute ammonia in excess. Alternative methods of effecting solution of the alkaloidal residue in standard acid were also tried, *viz.* (*a*) dissolving in 2 ml. of absolute alcohol before adding the standard acid, and (*b*) dissolving the residue in chloroform and boiling off the chloroform in the presence of the standard acid. The results of these further tests on the same sample were as follows:

Member	Conditions	Alkaloids: by titration Per Cent.	Alkaloids: by weighing Per Cent.
A	Slight excess of ammonia	0·49	—
	,, ,, ,, ,,	0·51	—
	5 ml. ,, ,, ,,	0·51	—
	Residue dissolved in alcohol	0·49	—
B	Slight excess of ammonia	0·48	—
	Residue dissolved in alcohol	0·48	—
C	Slight excess of ammonia	0·47	0·60
	5 ml. ,, ,, ,,	0·49	0·61
	Residue dissolved in alcohol	0·50	0·62
	,, ,, ,, chloroform	0·49	0·62
D	Slight excess of ammonia	0·46	—
	5 ml. ,, ,, ,,	0·48	—

As far as these results are significant, it appears that the results are slightly higher when an excess of ammonia is added. The method of dissolving the residue does not seem to have any effect. It is considered that these results are sufficiently satisfactory to recommend the method for adoption.

RECOMMENDED METHOD.—Introduce 10 g. of the lobelia in No. 60 powder and 10 g. of ignited sand into a pear-shaped separating funnel provided with a plug of cotton wool in the tube below the stopcock. Add 75 ml. of a mixture of 4 vols. of ether and 1 vol. of 95 per cent. alcohol (by volume). Shake and set aside for 15 minutes, add 5 ml. of dilute ammonia (10 per cent. of NH_3) and shake

in a mechanical shaker for 1 hour or by hand for 1 minute at 10-minute intervals during 1 hour. Allow the liquid to percolate into another separating funnel. When the liquid ceases to flow, pack the drug firmly by means of a button-ended glass rod. Continue the percolation, first with 25 ml. of the ether-alcohol mixture and then with ether, until the alkaloid is completely extracted, as shown by testing in the usual manner.

To the percolate add 30 ml. of *N* sulphuric acid.* Shake well and allow to separate. Run off the lower layer into another separator. Repeat the extraction with a mixture of 25 ml. of 0·5 *N* sulphuric acid with 5 ml. of alcohol (95 per cent.). Run off the lower layer and repeat with three further quantities of 20 ml. of the acid-alcohol mixture or until the alkaloids are completely extracted. Wash the mixed acid solutions, first with 10 ml. and then with successive quantities of 5 ml. of chloroform, washing each chloroformic solution with the same 20 ml. of 0·5 *N* sulphuric acid contained in another separator. Reject the chloroform, transfer the acid liquid from the second separator to the first separator, neutralise to litmus with dilute ammonia solution and add a further 5 ml. in excess.

Extract the alkaloids by shaking with successive quantities of 10 ml. of chloroform. Combine the chloroform solutions and wash with 3 ml. of distilled water. Filter the chloroform solution through a 7-cm. filter paper into a flask. Wash the filter thoroughly with more chloroform and collect the washings in the flask. Distil the chloroform from a water-bath until about 2 ml. remain. Add 2 ml. of absolute alcohol and continue the evaporation on the water-bath, using a gentle air-blast to complete the process. Repeat with two further lots of absolute alcohol to ensure dehydration of the residue. Heat the residue for 1 hour at 80° C.

Add to the residue 2 ml. of alcohol (95 per cent.) and warm until dissolved. Add 10 ml. of 0·02 *N* sulphuric acid. Cool and titrate with 0·02 *N* sodium hydroxide or sodium borate solution, using methyl red as indicator. One ml. of 0·02 *N* sulphuric acid is equivalent to 0·00674 g. of the alkaloids of lobelia calculated as lobeline.

A further sample of the drug was circulated, and the recommended method was compared with the methods of the French Codex (6th edition) and of the Swiss Pharmacopoeia (5th edition).

The results are set out in the following table:

	Lobeline on the undried drug		
Member	Recommended method Per Cent.	French Codex Per Cent.	Swiss Pharmacopoeia Per Cent.
A	0·351	0·345	0·405
	0·344	0·320	0·337
B	0·330	0·362	0·318
	0·320	0·342	0·320
	0·320	—	0·300
C	0·35	0·41	—

It was agreed that the recommended method gave more consistent results and is less open to objection than either of the two pharmacopoeial methods.

The experimental work reported in the tables was carried out by an appointed panel which had also the assistance of Mr. G. R. Page, of the British Pharmacopoeia Commission's Laboratory, in carrying out some of the analyses.

(*Signed*) G. ROCHE LYNCH (*Chairman*)
NORMAN EVERS (*Hon. Sec.*)

June, 1939

* It is important that hydrochloric acid should not be used, since lobeline hydrochloride is soluble in chloroform.

Notes

The Editor wishes to point out that the pages of the Journal are open for the inclusion of short notes dealing with analytical practice and kindred matters. Such notes are submitted to the Publication Committee in the usual manner.

A SIMPLE ELECTRICAL APPARATUS FOR HEATING SINGLE CRUCIBLES

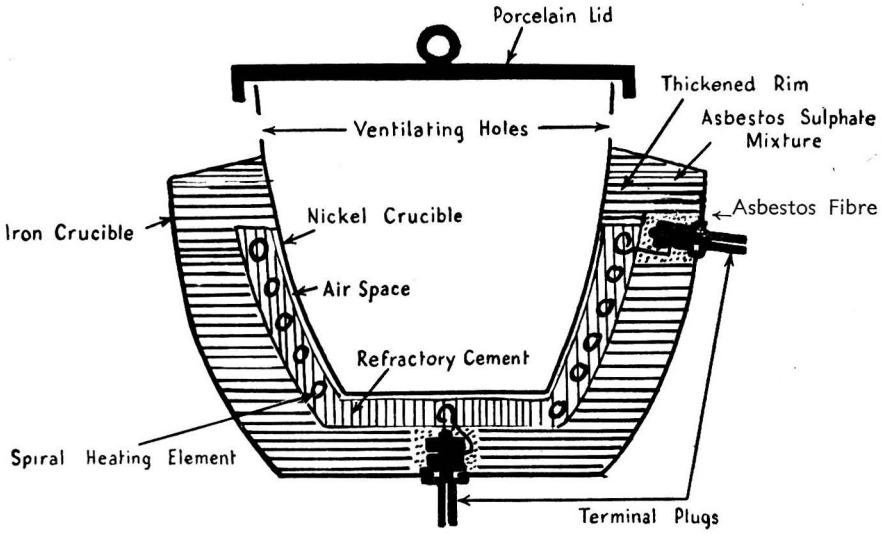
INCINERATION is a procedure frequently employed in biochemical research. Electrical or gas muffle furnaces as at present available are heavy, clumsy and expensive, and inability to control temperature to any degree of accuracy renders incineration by ordinary gas-burners unsatisfactory. For these reasons an economical, small, electrically heated furnace has been evolved which may be cheaply and easily constructed. The furnace holds one crucible and can be so designed that the internal temperature can be fixed at a predetermined level or varied to suit the operator's requirements.

The materials required for one furnace are:—(1) An iron crucible 5 in. (12·5 cm.) top diameter and 3 in. (7·5 cm.) deep. (2) A nickel crucible 3 in. (7·5 cm.) top diameter and 3 in. (7·5 cm.) deep. (3) Nickel-chrome resistance wire, 26 s.w.g., about 6 ohms/yard ("Brightray," Messrs. H. Wiggin & Co., Birmingham). (4) Two mica-insulated terminal plugs. (5) Two socket connectors to fit (4). (6) Asbestos wool, about $\frac{3}{4}$ lb. (330 g.). (7) Calcium sulphate (plaster of Paris) about $\frac{1}{4}$ lb. (110 g.). (8) Refractory cement about $\frac{1}{2}$ lb. (220 g.) ("Pyruma," Messrs. J. H. Sankey, London). The furnace is constructed in the following way:—Make a dry mixture of the asbestos and the calcium sulphate and knead about three quarters of it with water to the consistence of dough. Next take the iron crucible and press into it enough of the wet mixture to cover the bottom to a depth of $\frac{3}{8}$ in. (0·93 cm.), leaving a bare circular patch 1 in. (2·5 cm.) in diameter in the centre of the bottom. On this layer place a mould of the same shape as the iron crucible but with a top diameter of $3\frac{1}{2}$ in. (8·5 cm.). A small iron crucible makes a suitable mould. Pack the space between the mould and the crucible with the asbestos-sulphate mixture to within $\frac{1}{4}$ in. (0·6 cm.) of the top of the crucible, leaving another bare area 1 in. (2·5 cm.) in diameter 1 in. (2·5 cm.) from the rim. As soon as the packing has had time to harden a little the mould may be removed and the lining allowed to dry out. Drill holes $\frac{1}{4}$ in. (0·6 cm.) in diameter in the centre of the two areas of uncovered metal. These are for the terminal plugs.

It is necessary at this stage to take into account the work that the finished apparatus will be required to do. If it is to be used for a purpose requiring a fixed temperature it is necessary to arrange the resistance of the heating element accordingly (see below). If the apparatus is to be used at more than one temperature, one of several alternative arrangements may be employed. The most economical of these is to construct two furnaces and to connect them in series across the mains so that the temperature produced is the lowest at which ashing is likely to be carried out. This allows subsequent alterations of temperature by re-arrangement of the connections and addition of a suitable rheostat. Another method is to make one furnace only and use it with a rheostat in series. This last method is rather wasteful, because power is unavoidably lost in heating the rheostat. Also, the rheostat alone costs as much as the furnace itself.

If one apparatus only is required to work at a fixed temperature which will be high enough to give complete oxidation of carbon without loss of potassium or other relatively volatile elements, that is, approximately 450° C., the total resistance of the windings should be about 100 ohms at 220 volts, and the heating element will accordingly consist of 15 to 18 yards (metres) of the nickel-chrome

resistance wire. If two furnaces are to be made and used for ashing at 450° C. they should each have a resistance of about 45 ohms at 220 volts and be connected in series. If a higher temperature is required, the two can be disconnected and either of them connected in series with a rheostat, which should have a total resistance of 50 ohms, and be capable of carrying 5 amps. at 220 volts.



Having decided on the most suitable arrangement, measure off the required length of wire and wind it on a rod into a close spiral of about 3/16 in. (0·5 cm.) diameter. When all the wire is wound, draw out the spiral to a length of about 3 feet (1 metre). Straighten both ends of the wire for a distance of 1 in. (2·5 cm.). Then wet thoroughly the inner surface of the asbestos and apply a layer of refractory cement about $\frac{1}{8}$ in. (0·3 cm.) thick. Pass one end of the spiral of wire through the hole in the bottom of the crucible, fix it with a cork and then partly embed the wire in the refractory cement in a regular spiral formation. Pass the other end of the wire through the hole near the rim of the crucible and secure it with another cork. Allow the whole crucible and its contents to dry in an oven at 100° C. When dry, insert the mica-insulated plugs into their holes and fasten down the securing nuts. Connect the ends of the resistance wire with their respective terminals and screw down the locking nuts to hold the wire. Fill in the space around the nuts with wet asbestos fibre until level with the inner wall of refractory cement. This soft filling facilitates subsequent removal of the wire should it become necessary. Now cover the whole inner surface of the crucible and the coils of wire with a layer of refractory cement. The total thickness of cement will now be about $\frac{1}{4}$ in. (0·6 cm.); the coils of wire should be just covered and the inner surface smooth.

Drill 9 holes, $\frac{1}{4}$ in. (0·6 cm.) in diameter, in a ring $\frac{1}{2}$ in. (1·2 cm.) from the rim of the nickel crucible and place the nickel crucible inside the asbestos-lined iron crucible so that it clears the refractory lining by about $\frac{1}{8}$ in. (0·3 cm.) both from sides and bottom (see sectional diagram). It may be held off the bottom at this stage by a small piece of packing material. Then fill the space between the two crucibles above the embedded heating element with the same asbestos-sulphate mixture as was used for the preliminary lining. The position and shape of this thickened rim can be seen from the diagram. As soon as the rim has set take out the nickel crucible and remove the temporary packing piece. When the whole

is thoroughly dry replace the nickel crucible, which should now be held firmly by the thickened upper rim.

The furnace may conveniently be mounted in a 5½ in. (14 cm.) ring clamped on a retort stand. When in place plug in the socket connectors fitted to 5-amp. asbestos flex and cover the nickel crucible with a porcelain lid 3½ in. (8·75 cm.) in diameter. The furnace is now ready for testing. This should be done under working conditions with a 500° C. thermometer. If necessary, the temperature may be reduced by means of a small external resistance, consisting of a few turns of resistance wire wound on a piece of uralite. The material to be ashed is put into a silica or platinum crucible within the furnace. The apparatus will be found to ash satisfactorily at any temperature between 400 and 450° C., and the time taken to oxidise all the carbon will vary from 1 to 36 hours, depending on the type of material being incinerated.

It is clear that this apparatus has many applications other than that for which it was originally designed (dry ashing at 450° C.). Thus it may be used as a heating unit for distilling small quantities of liquids, and for this purpose the nickel crucible can be partly filled with a liquid of suitable boiling-point, with sand or with flake asbestos, or may be removed entirely. The apparatus may also be useful in the determination of melting-points. Units that were constructed to run at a temperature of 450° C. on a 200-volt supply have been found to make excellent heaters for Soxhlet extractions (ether). For this purpose the nickel crucibles were removed and the terminals were connected with a 100-volt source of current.

Furnaces as used in this department may be obtained from the Scientific Glassblowing Co., Ltd., London.

SUMMARY.—The construction of a simple inexpensive apparatus is described for dry ashing at 450° C. Simple modifications allow the temperature to be varied to suit other requirements and in this way the apparatus may be used for other purposes.

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MOHLER'S TEST FOR BENZOIC ACID

A DISADVANTAGE of Mohler's test, described in THE ANALYST (1932, **57**, 224) is the rapid fading, on dilution, of the colour due to *m*-diamino-benzoic acid. It has recently been discovered that if the dilution is made with the solution prepared as described below, the colour does not fade and the mixture may be diluted at will.

Preparation.—Twenty ml. of conc. sulphuric acid are added to 40 ml. of water containing 2 g. of potassium nitrate, and the liquid is cooled while 200 ml. of water containing 100 ml. of ammonia (sp.gr. 0·880) are carefully added. Finally, 40 ml. of water, in which 0·8 g. of hydroxylamine hydrochloride is dissolved, are added to the solution.

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THE DETERMINATION OF MANGANESE, ESPECIALLY IN BIOLOGICAL MATERIAL

OXIDATION of manganese by means of periodate has been found satisfactory for the determination of the metal in a wide variety of materials; according to the amount of manganese present, the determination may be completed colorimetrically or volumetrically. The process is carried out in a hot mixture of phosphoric and sulphuric acids. (*Cf.* Willard and Greathouse, *J. Amer. Chem. Soc.*, 1917, **39**, 2366; Abst., ANALYST, 1918, **43**, 44; Richards, ANALYST, 1930, **55**, 554.) For quantities of manganese between 0·1 and 1·0 mg. it has been found

possible to measure photoelectrically the permanganic acid formed. The instrument we have used is the Hilger Spekker Absorptiometer. The excess of the oxidising agent in the reaction mixture stabilises the permanganate formed, so that the solution is suitable for colorimetric measurement. With lead peroxide or sodium bismuthate as oxidising agent, this advantage is not present.

We have found the method to be satisfactory for the determination of manganese in biological material after it has been ashed. With pharmaceutical products containing large amounts of iron the method also works well; the procedure is to destroy the organic matter by ignition, to fuse the residue with potassium bisulphate, and to oxidise the manganese; excellent results have been consistently obtained. Appreciable amounts of manganese have been found in a number of commercial samples of iron salts, as shown below:

Ferrous sulphate (AnalaR)	100 p.p.m.
Exsiccated ferrous sulphate (Coml.) ..	700 ,,
Iron ammonium citrate (scales B.P.) ..	300 ,,

When the quantities of manganese lie between 10 and 30 mg. we use a slight modification of the volumetric method put forward by Willard and Thompson (*Ind. Eng. Chem., Anal. Ed.*, 1931, 399; Abst., ANALYST, 1931, **56**, 830). The excess of periodate, after completion of the oxidation, is precipitated with mercuric nitrate; the reaction mixture is filtered into a known amount of ferrous sulphate solution, sufficient to furnish an excess that can be determined with permanganate. Willard and Thompson used "$Hg(NO_3)_2 2H_2O$ dissolved in a little water" for precipitating excess of periodate. We examined two samples of mercuric nitrate (obtained in this country) and found them to be basic and only partly soluble in water. We therefore dissolved the requisite amount for precipitation in the minimum necessary quantity of dilute nitric acid, and oxidised this solution with just sufficient permanganate; it was then ready for use as a precipitant. We found that asbestos, recommended for the filtration by Willard and Thompson, caused a small loss of manganese, and were able successfully to replace it by a layer of kieselguhr ("Hyflo Supercel"). We have also found that it is possible to carry out the oxidation in dilute sulphuric acid (5 per cent. w/v) without any phosphoric acid being present, and that this does not lead to trouble from precipitation of manganese oxides. We have applied the method to manganese sulphate, to manganese butyrate after removal of butyric acid by evaporation with sulphuric acid, and to manganese glycerophosphate after ignition. We have found that the B.P.C. method for assaying manganese glycerophosphate by ignition to pyrophosphate may give misleading results; commercial samples apparently contain from 1 to 3 per cent. of calcium, and this is calculated as manganese in assays by the B.P.C. method. It is better to dissolve the ash, after ignition, in dilute sulphuric acid, and to determine the manganese by the method of Willard and Thompson.

In attempting to determine manganese in manganese sulphate by precipitation as manganese ammonium phosphate, we obtained variable results. Satisfactory results, on the other hand, were obtained by the method of Volhard (Treadwell and Hall, 4th Ed., Vol. II, p. 612), in which manganous manganese is titrated with potassium permanganate in hot solution in presence of zinc sulphate. The end-point of this method, however, is difficult to ascertain, owing to the presence of a brown precipitate of zinc manganite.

I would like to express my thanks to Mr. A. F. Lerrigo for his advice and criticism of the work that forms the basis of this note, and to the Directors of Glaxo Laboratories, Ltd., in whose Analytical Department it was carried out, for permission to publish the results.

F. E. READ

GLAXO LABORATORIES, LTD.
GREENFORD, MIDDLESEX

June 21st, 1939

NOTES ON THE VOLUMETRIC IODIDE METHOD[1] OF DETERMINING STARCH

THE convenience and rapidity of centrifugal methods in the separation of interfering substances is shown in the following description of the analysis of cocoa. The starch in one sample can be determined in 1½ hours and the only multiplication of apparatus needed for the completion of 20 samples in a day is that of the centrifuge tube. The method is capable of general application to starch-containing substances and may usually be shortened. Thus there is no need for a preliminary separation of sugar with diluted alcohol unless it is present in sufficient quantity to produce large amounts of caramel when heated with alcoholic potassium hydroxide; the heating with alcoholic potassium hydroxide can be omitted when only small amounts of fat and protein are present; it is unnecessary to re-precipitate the starch with 40 per cent. alcohol and wash with $N/5$ potassium acetate solution in the absence of dextrin, and the results for starch are within 0·1 per cent. if this is omitted in chocolate analysis. The presence of colouring matter, tannin and other substances in cocoa, which are precipitated if an attempt is made to neutralise the solution, before adding iodine to precipitate the starch, renders a preliminary separation necessary so that these substances can be removed by decantation and washing before the final titration for starch, and this is best done by adding iodine to the aqueous potassium hydroxide solution. If quantities larger than 0·5 g. of cocoa are used the decanted solution should be tested to see that it contains an excess of iodine.

The presence of fibre in cocoa facilitates the clarification of the various solutions, which can be decanted without filtration. A volumetric measure of the fibre is obtained if, after acidifying the final titrated starch and fibre mixture (with 3 ml. of conc. hydrochloric acid), the tube is heated in a boiling water-bath and then whirled; after the clear liquid has been decanted the fibre can be washed in the centrifuge tube, transferred to a platinum dish, dried and weighed. Whirled for 5 minutes at a speed of 1000 r.p.m., 1 ml. of apparent fibre = approx. 0·02 g. of dried fibre.

In my opinion the determination of starch means the determination of the amylo group, whether it is present as amylose, amylopectin or amylo-hemicellulose, but the last of these may be approximately estimated by adding taka-diastase to the gelatinised mixture at *p*H 7 and incubating at 38° C. for 1 hour; the amylose and amylopectin are transformed into sugar, leaving the insoluble amylohemicellulose, the amylo group of which can then be determined by the iodide method.

STARCH IN COCOA.—The preliminary treatment for the removal of interfering substances yields a mixture of starch and fibre; the starch is then determined by the volumetric iodide method.

(*a*) *Removal of fat and protein.*—Weigh 0·5 g. of cocoa into a 50-ml. centrifuge tube, add 30 ml. of an 8 per cent. alcoholic solution of potassium hydroxide and heat (with stirring) for 5 minutes in a water-bath at 80° C.; dilute the mixture to 50 ml. with industrial spirit and whirl in a centrifuge; decant the clear liquid and wash the residue once with hot industrial spirit, whirl and decant the clear liquid.

(*b*) *Preparation of starch solution.*—To the residue from (*a*) add 20 ml. of a 0·7 per cent. aqueous solution of potassium hydroxide and heat (with stirring) for 10 minutes in a water-bath at 95 to 100° C.; dilute the mixture to 40 ml. with water and cool.

(*c*) *Initial precipitation of starch to remove colouring matter, etc.*—To the starch solution from (*b*) add 4 ml. of N iodine solution and adjust to 50 ml. with approximately 2 N potassium acetate solution, cork the tube, shake, whirl and decant the clear liquid; wash the residue once with approximately $N/5$ potassium acetate solution and 1 ml. of $N/10$ iodine solution, cork the tube, shake, whirl and decant the clear liquid.

(*d*) *Re-precipitation of starch iodide.*—To the residue from (*c*) add 2 ml. of *N*/10 sodium thiosulphate solution (to reduce the iodide and re-dissolve the starch), dilute the mixture to 28 ml. with water, add 2 ml. of *N*/10 iodine solution and 20 ml. of industrial spirit, cork the tube, shake, whirl and decant the clear liquid; wash the residue once with approx. *N*/5 potassium acetate solution and 1 ml. of *N*/10 iodine solution, cork the tube, shake, whirl and decant the clear liquid.

(*e*) *Volumetric determination of starch.*—To the residue from (*d*) add 1 ml. of *N*/10 sodium thiosulphate solution to dissolve the starch, dilute to 40 ml. with water, add 4·5 ml. of *N*/10 iodine solution followed by 5 ml. of approx. 2 *N* potassium acetate solution, make up to 50 ml. with water, cork the tube, shake and whirl for 10 minutes. Pipette off 25 ml. of the clear liquid and titrate with *N*/100 sodium thiosulphate solution; titrate the remaining liquid together with the precipitate with *N*/100 sodium thiosulphate solution, and calculate the starch.

STARCH IN SWEETENED CHOCOLATE.—(1) *Removal of sugar.*—Weigh 2 g. of chocolate into a 50-ml. centrifuge tube, add 15 ml. of hot water and, after stirring to dissolve the sugar, add industrial spirit and 1 ml. of *N* iodine solution to the 50-ml. mark; cork the tube, shake, whirl and decant the clear liquid; wash the residue once with 70 per cent. industrial spirit, cork the tube, shake, whirl and decant the clear liquid.

(ii) *Determination of starch.*—To the residue add 30 ml. of an 8 per cent. alcoholic solution of potassium hydroxide and proceed as for cocoa.

RESULTS.—The following results were obtained:

	Starch in original substance Per Cent.	Starch in fat-free and sucrose-free dry substance Per Cent.
Cocoa A	11·0	15·0
,, B	11·2	16·0
,, C	12·7	17·5
,, D	13·0	18·0
Chocolate E ..	2·2	16·0
,, F ..	2·3	16·0
,, G ..	2·5	16·5
,, H ..	2·9	17·0

The fat was extracted from Cocoa C, the fat-free substance was dried, and the sample was well mixed. Four 0·5-g. samples were weighed into centrifuge tubes, and the analysis was carried out as described. The titrated mixtures of starch and fibre were acidified with 3 ml. of conc. hydrochloric acid, and the tubes were placed in a boiling water-bath for 30 minutes and then whirled; after the acid liquid had been decanted, the fibre was washed twice with water, transferred to a platinum dish, dried and weighed and ignited, and the ash was weighed.

The following results were obtained:

Number of test	Weight of fat-free dried substance g.	Starch found by volumetric iodide method Per Cent.	Apparent volume of fibre ml.	Weight of dried fibre g.	Weight of ash in dried fibre g.	Fibre Per Cent.
1	0·5	17·6	1·4	0·031	0·002	5·8
2	0·5	17·5	1·4	0·032	0·002	6·0
3	0·5	17·5	1·4	0·032	0·002	6·0
4	0·5	17·4	1·4	0·031	0·002	5·8
Average	0·5	17·5	1·4	0·0315	0·002	5·9

Fibre determined by the usual method of digesting the dry fat-free material with dilute sulphuric acid and then with alkali:

2·0	—	—	0·119	0·003	5·8

The taka-diastase method was applied to the titrated starch and fibre mixtures. Five ml. of a 1 per cent. solution of taka-diastase were added to each tube, the *p*H was adjusted to 4 with sodium acetate and acetic acid buffer, including that of the blank, the tubes were incubated at 38° C. for 36 hours, and the resulting sugars were determined by the ferricyanide and iodine method; the polarimetric and copper reduction method was also used, by re-precipitating the starch from a number of titrated solutions and collecting the starch and fibre into one tube so as to obtain a larger reading on the polarimeter. The starch found by both methods was within 0·5 per cent. of that determined by the iodide method. The average amount of starch in the fat-free dried substance of cocoa, found by the usual taka-diastase method, is about 14 per cent.

The explanation of this low result with the taka-diastase method probably lies in the fact that insoluble compounds of starch, such as amylo-hemicellulose, are only slowly acted on by taka-diastase at a *p*H above 6. On applying the iodide method to the insoluble residue left after the usual taka-diastase action on the fat-free dried substance had proceeded for 24 hours, it was found that 1·5 per cent. of starch remained in the residue, and a duplicate test after 48 hours' incubation showed that 0·5 per cent. remained in the residue. Similar results are obtained with some cereals unless the *p*H is adjusted to 4 or 5. If the *p*H is adjusted to 4 with a buffer of sodium acetate and acetic acid the addition of toluene is unnecessary.

It has been found that the following table of factors applies to all quantities of starch if 4·5 ml. of *N*/10 iodine solution (containing 0·25 g. of potassium iodide) and 5 ml. of 2 *N* potassium acetate solution of *p*H 6·4 (adjusted with acetic acid) are used in the test:

N/10 iodine in excess in 50 ml. ml.			Starch g.
4·0	1 ml. of *N*/100 sodium thiosulphate	=	0·00555
3·5			0·00561
3·0			0·00571
2·5			0·00582
2·0			0·00592
1·0			0·00643

Reference

1. W. Whale, Analyst, 1938, **63**, 328, 421.*

W. Whale

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June 1st, 1939

ASSAY OF BELLADONNA ROOT AND ITS PREPARATIONS

The B.P. method for the assay of belladonna root does not work satisfactorily with "Atropa Belladonna" obtained from Kashmir. On account of the abnormally large amount of resins and red colouring matter found in these roots (which consist mostly of the "crowns" rather than the longitudinal stems of the roots) as compared with the official roots, the final alkaloidal residue is so highly coloured that it is impossible to titrate it accurately. The same difficulty is experienced in the assay of preparations from these roots.

* Copies of this reprint may be obtained on application to the author.

Gum tragacanth has been found by a number of workers to eliminate most of the troublesome resins and colouring matter which interfere with the assay of alkaloidal drugs; I have found that the use of gum tragacanth gives excellent results in the assay of belladonna root and its preparations. The actual manipulation is as follows:

ASSAY OF BELLADONNA ROOT.—The alkaloids are liberated and extracted with ether and alcohol as described in the B.P. Very often about 400 to 600 ml. of percolate is obtained for every 10 g. of root powder, and this may advantageously be partly concentrated over a warm water-bath. After cooling, 1·0 g. of gum tragacanth powder and 2 to 3 ml. of water are added to the percolate and thoroughly shaken for about five minutes. The gum coagulates readily, removing most of the resins and colouring matter from the alkaloidal solution. The solution is allowed to stand for about five minutes—when it will be clear—and filtered into a separating funnel through a tight plug of cotton wool, the flask and the residue being washed with small portions of ether until the alkaloids have been completely transferred to the separator. The assay is then continued with this solution, as described in the B.P., but with the use of dilute hydrochloric acid instead of sulphuric acid for extraction.

ASSAY OF PREPARATIONS FROM BELLADONNA ROOT.—Ten ml. of liquid extract (or corresponding quantities of other preparations) are mixed with 1·0 g. of tragacanth powder and 1·5 to 2·0 ml. of dilute solution of ammonia are added. The mixture is thoroughly stirred with a glass rod until the gum coagulates. The alkaloids are completely extracted with ether by kneading the coagulated mass in the solvent, each portion of ether being filtered into a separator through a tight plug of cotton-wool. The assay is then carried out as indicated above.

The advantages of this modified procedure are:

1. The formation of troublesome emulsions is completely avoided and the extractions can be carried out more quickly. The assay can be completed in about 8 or 9 hours, whereas the B.P. method requires 12 to 14 hours.

2. The final alkaloidal residue is obtained as a light brown, transparent mass giving a colourless or very pale yellow solution in sulphuric acid which can be titrated much more easily and accurately than the deep reddish solution obtained by the B.P. method.

3. The method can be applied to all commercial grades of "Atropa Belladonna," even those that contain unusually large proportions of resins and colouring matter.

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June 24th, 1939

Notes from the Reports of Public Analysts

The Editor would be glad to receive the Annual or other Reports of Public Analysts containing matter of special interest to the Society. Notes made from such Reports would be submitted to the Publication Committee.

CITY OF BIRMINGHAM

Report of the City Analyst for the First Quarter, 1939

Of the 1541 samples submitted under the Food and Drugs Act, 1475 were bought informally.

Formalin Tablets without Formalin.—Three samples were returned as incorrect. According to the B.P. Codex each tablet should contain 9·7 mg. of paraformaldehyde together with citric acid, menthol and a little oil of lemon. The first sample consisted of three different kinds of tablets: about 20 large tablets containing no formalin at all, three small opaque tablets, which also contained no formalin, and three small translucent tablets, each of which contained 4 mg. of paraformaldehyde. The vendor was aware that there were three different kinds, but had bought them in good faith from a reputable firm. This firm stated that the tablets they supplied were not of B.P.C. strength, but should have contained 4·7 mg. of paraformaldehyde per tablet; in future all their tablets would be made to the B.P.C. standard.

The second sample contained only 4 mg. of paraformaldehyde per tablet. The wholesale dealer collected the whole stock and destroyed it. The third sample contained only 2 mg. of paraformaldehyde per tablet. In this case the wholesale dealers, replying to the vendor, said that, whilst there was a deficiency of formalin, the tablets sent to them were a mixed lot and some were not of their manufacture. They also alleged that it was difficult to guarantee that the full amount of formalin would be retained over a prolonged period unless the tablets were kept in completely air-tight containers and not exposed to warmth. The retailers advised all their branches to carry very small stocks and to exercise special care in storage. Tests applied to the stock of tablets at headquarters showed them to be of full strength.

H. H. Bagnall

CITY OF SALFORD

Annual Report of the City Analyst for the Year 1938

Of the 1284 samples examined, 396 were bought formally.

Cheshire Cheese.—A sample purchased as "full cream Cheshire cheese" was found to contain 29·0 per cent. of fat and 44·8 per cent. of moisture (= 52·5 per cent. of fat on the dry substance). Enquiries by the sampling officer showed that the sample was a full cream cheese of Dutch origin. As there is no Marking Order under the Merchandise Marks Act, 1926, requiring cheese to be labelled, and as it is acknowledged that Cheshire type cheese can be manufactured outside this country, and, further, since the sample conformed to the standard of 45 per cent. of fat on the dry cheese, no action could be taken against the vendor. The Cheshire Cheese Federation propose to take joint action with other British cheese interests with a view to applying for a Marking Order.

Potassium Iodide Method of Measuring Solar Radiations.—This process has been in continuous use for 12 years at four stations (*cf.* Analyst, 1933, **58**, 690; 1937, **62**, 667). The solar radiation is expressed as milligrams of iodine

liberated by an exposure from 9 a.m. to 9 a.m. The totals for 1938 and the yearly averages for 5 years were as follows:

	Regent Road	Nab Top Sanatorium, Marple	Ladywell Sanatorium	Drinkwater Park
Year's total ..	1786·0	2703·0	2294·6	2208·8
Average ..	1683·8	2426·4	2002·8	1953·0

The highest figures were, of course, obtained at Marple, where the atmosphere is much clearer than in Salford.

Attention has previously been directed to the fact that once the potassium iodide solution has become coloured with iodine the speed of the reaction, *i.e.* the amount of iodine liberated slows down rapidly; this can be demonstrated either by adding a known quantity of iodine in a duplicate test, when the amount of iodine liberated on exposure will be less than in the test as ordinarily carried out, or by placing bottles out at short intervals (say 2 hours), when the total amount of iodine liberated will be greater than usual. In order to get further information bearing on, and also possibly to arrive at some quantitative idea of the extent of, this slowing down of the reaction, it was decided to expose a solution of potassium iodide to the light transmitted by various glass filters. For this purpose, eight different types of Chance–Parsons colour filters were used, the potassium iodide solution being held in transparent quartz test-tubes placed behind the filters in such a manner that the only light reaching the tubes was that transmitted by the filters. The effect of a piece of ordinary window glass was also determined. Exposures were made at exactly the same time towards the same portion of the sky, and were of about seven hours' duration, the contents of the tubes being then titrated immediately. The following table gives the average figures for five series of tests:

EFFECT OF CHANCE–PARSONS FILTERS ON POTASSIUM IODIDE TEST

	Blank	Window-glass	"Daylight" glass	Blue-green	Ultra-violet	Purple	Light orange	Dark orange	Ruby	Red
Average amount of iodine liberated (5 tests), mg. ..	2·57	2·32	2·26	1·72	0·97	0·73	0·25	0·11	0·07	0·07
Percentage of blank	—	90·3	87·9	66·9	37·7	28·4	9·7	4·3	2·7	2·7

The most striking feature of these results is the fact that the highest figures were obtained when a proportion of both U.V. and visible blue rays were transmitted by the filters, *i.e.* quartz, window-glass, "Daylight" glass and blue-green filter. In the next group come the U.V. and purple filters, both of which transmit a proportion of the U.V. rays, but practically no visible blue rays. Lastly we have the four orange and red filters, which transmit neither U.V. nor visible blue rays, and which all slow down the reaction by at least 90 per cent. In more precise terms, quartz transmits all rays down to about 200$m\mu$ and window-glass down to about 320$m\mu$. The figures supplied by the makers of the filters indicate that the "Daylight" filter transmits down to about 328$m\mu$, the blue-green between 370$m\mu$ and 588$m\mu$, the ultra-violet between 313$m\mu$ and 400$m\mu$ with some transmission above 700$m\mu$, and the purple between 345$m\mu$ and 410$m\mu$ with some transmission above 690$m\mu$. The four orange and red filters do not transmit rays below 500$m\mu$.

There is relatively little difference between the results obtained with quartz, and with quartz and window-glass, suggesting that the shorter U.V. rays do not play a large part in the reaction; on the other hand, the effect of even the light orange filter is very great. Although the experimental accuracy of work with colour

filters is somewhat questionable, owing to the difficulty that the various wavelengths overlap, the experiments so far carried out indicate that this reaction is due almost entirely to wavelengths between 300$m\mu$ and 500$m\mu$.

ASHWORTH'S ULTRA-VIOLET RAY METER.—It has been found that the ordinary photographic paper used in this instrument (*cf.* ANALYST, 1933, **58**, 690; 1937, **62**, 667) varies considerably in sensitivity, and therefore potassium dichromate light-sensitive paper was used instead for the observations during the last 10 months of the year.

INTEGRATING SOLARIMETER.—So far as I am aware, only one other instrument of this type is in use in this country, and that is a recording solarimeter at the Meteorological Office, South Kensington. The instrument is essentially a Moll pattern thermopile coupled with a sensitive milliampere-hour-indicator. The solar radiation is therefore transformed into and recorded as, electrical energy, the apparatus having previously been calibrated by exposure to a standard source of radiation. The results are in terms of absolute units of energy and give the total solar radiation, both visible and invisible. The results recorded by the Salford and Kensington instruments (the latter results kindly supplied by the Director of the Meteorological Office) were as follows:

SOLARIMETER—DAILY MEAN RADIATION

(Gramme Calories per Square Centimetre)

Month	Salford	South Kensington
January	11·33	38·5
February	26·55	75·6
March	85·50	160·0
April	122·25	227·0
May	190·50	281·0
June	222·25	377·4
July	177·08	283·0
August	168·75	234·0
September	88·95	185·9
October	48·75	106·0
November	18·00	60·4
December	7·73	30·1

The results show pronounced differences between the two series of observations, especially in the winter months, and they indicate in a striking manner the very considerable screening of daylight by smoke clouds in the air above this City.

G. H. WALKER

Lagos

ANNUAL REPORT ON THE DEPARTMENT OF CHEMISTRY FOR 1938

THE work of the Department of Chemistry (under the direction of Mr. C. L. Southall, B.Sc., A.I.C.) is mainly concerned with the examination of samples and exhibits submitted by various Departments, notably Customs, Medical and Health, and Police. The total number of examinations made during the year was 2615, which was a small increase on the previous year. A large proportion of the work related to water supplies, and involved 912 bacteriological and 172 chemical examinations.

TESTS FOR *B. COLI*.—The authors of the Ministry of Health Bulletin, No. 71, requested water bacteriologists to test Levine's Eosin—Methylene Blue—agar in comparison with the usual MacConkey's neutral red agar, which is not very satisfactory in the tropics. Extensive tests indicated that it was possible when

using Levine's agar to distinguish between typical *B. coli* and *B. aerogenes* by direct inspection after incubation for 24 hours at 37° C. The use of this medium has been adopted and is particularly helpful when examinations have to be made on the spot.

PRECIPITIN TEST FOR BLOOD ON WATER-PROOFED MATERIAL.—The precipitin test for blood was applied to 430 exhibits. In one most important case it failed. The prosecution suspected that a rubberised coat had been used to wrap up a recently severed head. There were stains of blood on the fabric, which had been partly washed out, but the precipitin test could not be applied because extracts from unstained portions of the coat all gave positive reactions. Two other raincoats were tested, and, while a heavily rubberised coat gave positive results, another raincoat did not do so. No method of separating the blood from the interfering proofing matter could be discovered, and advice on this point from other workers would be most welcome.

FLUORESCENCE OF SEMINAL STAINS.—There was an increase in the number of exhibits submitted in cases of alleged rape. In this connection it was noticed that some white stains on indigo-dyed woollen cloth showed no fluorescence in ultraviolet light, although perfect spermatozoa were readily extracted from them.

POISONING CASES.—There was a wider range of poisons than in former years and the following substances were isolated from viscera: Strophanthin, hyoscyamine, physostigmine (Calabar bean), thevetin, quinine, sasswood, croton oil, camphor, methyl salicylate, ammonia, caustic alkali, phenol, prontosil, cyanide, mercury and bismuth. In no case was arsenic found, but on several occasions toxic organic material which could not be identified was isolated.

HYDROCYANIC ACID IN LAGOS FOODSTUFFS.—The hydrocyanic acid content of various native foods was estimated colorimetrically by a modification of the method of Childs and Ball (ANALYST, 1935, **60**, 296). Whereas samples of raw material contained as much as 40 p.p.m. of CN, only one sample of prepared foodstuff contained as much as 1 p.p.m. In spite of this, many medical officers are convinced that cyanide poisoning is prevalent, and it is hoped that further samples will be submitted for examination.

COMPOSITION OF HUMAN MILK (AFRICAN).—A sample, taken under conditions that ensured its being genuine and average, contained 5·4 per cent. of fat and 8·8 per cent. of solids-not-fat. Similar abnormal figures have been reported in a few instances from other African Colonies.

NIGERIAN LIGNITE.—A sample was found to have a proximate composition almost identical with that of the brown lignite deposits in Germany.

Hong Kong

ANNUAL REPORT OF THE GOVERNMENT ANALYST FOR THE YEAR 1937

THE work of the Hong Kong Government Laboratory (under the direction of Mr. C. V. Branson, B.Sc., F.I.C.) differs from that usually associated with a subdepartment of this type, in that a considerable amount of work usually done by consulting chemists is done there, the fees being paid into the Treasury. Practically the whole of the official work is chemico-legal.

TOXICOLOGICAL EXAMINATIONS.—The total number of samples examined was 236 as compared with 199 in the previous year; poisons were detected in 164. Suicide again accounted for most of the deaths. Opium, detected in 46 cases, was still the most common agent, but a very large increase in poisons of the lysol type (39) was recorded. Arsenic derivatives were found in 25 cases, barbituric acid derivatives in 8, *Gelsemium elegans* in 8, santonin in 2, mercury in 2, lead in 2, and mydriatic alkaloids in 1.

BOMB OUTRAGES.—The Laboratory was consulted in connection with three bomb outrages. In one of these a parcel was delivered to a house by a private messenger, and, when an attempt was made to open it, it exploded and produced fatal injuries. After investigation of the pieces, it was possible to construct a copy of the bomb; this was produced in Court. The bomb was exploded by means of an electrical contact firing a charge of black powder and broken glass. In the other two cases the bombs were of the Mills type and were filled with a mixture of picric acid, perchlorate and aluminium.

COUNTERFEIT COINING.—There was a great reduction in the number of cases—11 as compared with 78 in 1936. The new nickel coins in circulation are much more difficult to counterfeit than the older coins, so that a further decrease is to be expected.

Fruit and Vegetable Preservation Research Station, Campden

ANNUAL REPORT FOR THE YEAR 1938

THE Director (Mr. F. Hirst, M.Sc.) reports that the feeding tests which have been conducted at the Rowett Institute, Aberdeen, over a period of two years (*cf.* ANALYST, 1938, **63**, 823) are now completed. The results, to be published shortly, do not show any definite difference in breeding data, lactation, growth and other factors between rats bred and fed on a diet consisting entirely of canned foods and those on a similar diet of the corresponding foods cooked in the ordinary way. They confirm the results obtained in France and the United States of America, and, when taken in conjunction with those of other workers on the vitamin and mineral salt content, indicate that the nutritional value of canned products compares favourably with that of home-cooked food.

The study of the distribution and losses of the various mineral constituents of certain vegetables during home cooking and canning, which was begun about the same time as the feeding experiments at Aberdeen, has also been completed, and the results are described in a section of this report. These and other health aspects of canned foods are being reviewed in collaboration with a member of the staff of the Rowett Research Institute, and it is hoped that a comprehensive bulletin on the subject will be ready for publication in the near future.

During the year under review 719 technical enquiries were answered by post and probably a greater number by telephone. In addition, 326 samples of canned products were examined and reported on, and 104 canner's thermometers and pressure gauges were tested. The subjects investigated at the Station included the following:

FRUIT-GUM IN PLUMS.—In the previous Report (*cf.* ANALYST, 1938, **63**, 823) it was suggested that the site at which surface spots of gum developed might be correlated with the presence or absence of internal gum. In the season under review it was noted that soft spots of gum developed very extensively on the distal ends of Victoria plums grown locally. Many of these spots hardened and fell off as the fruit ripened. Thirty-six of such plums were canned, and in thirty of them severe internal gumming was noted in the canned product.

DRAINED WEIGHT OF ENGLISH CANNED FRUIT.—The results of an investigation by the Director and W. B. Adam, in which the figures obtained during the past ten years were studied, indicated that the degree of ripeness, the strength of the syrup used, and the conditions of processing all have some influence on the drained weight; this also alters during storage. The wide difference between the average and minimum drained weights recorded makes it difficult to establish

standards based on drained weights. The texture of the canned fruit is of such importance in this connection that some reference to wholeness or firmness of the fruit would appear to be essential in framing appropriate regulations. Such regulations could better be based on the average drained weight of a definite number of cans of each commercial brand than on a minimum figure which applied to every can packed.

COMPOSITION AND CANNING PROPERTIES OF LOGANBERRIES AND BLACKBERRIES.—The primary object of this work by the Director was to compare two pairs of closely allied fruits—the *Loganberry* and the *Phenomenal Berry*, and *Black Diamond* and *Himalayan Giant* blackberries. The following table shows the chief characteristics of the two types of fruit canned in 1936, 1937 and 1938. The figures (except for 1938) are an average of all the results obtained. Acidity is expressed as anhydrous citric acid:

LOGANBERRIES AND PHENOMENAL BERRIES

	Composition of fresh fruit			Canned fruit	
	Acidity Per Cent.	Soluble solids Per Cent.	Insol. solids Per Cent.	*p*H	Loss in weight Per Cent.
1936					
Loganberries ..	2·80	8·8	5·6	2·85	14·5
Phenomenal Berries	2·85	8·8	5·7	2·82	15·1
1937					
Loganberries ..	2·56	9·5	5·8	2·96	12·3
Phenomenal Berries	2·56	10·1	5·6	2·88	12·1
1938					
Loganberries ..	2·74	11·0	5·1	2·76	15·3
Phenomenal Berries	2·72	10·9	4·9	2·77	17·9

BLACKBERRY VARIETIES

	Composition of fresh fruit			Canned fruit	
	Acidity Per Cent.	Soluble solids Per Cent.	Insol. solids Per Cent.	*p*H	Loss in weight Per Cent.
1936					
Black Diamond ..	2·00	9·0	4·6	3·03	10·1
Himalayan Giant ..	2·03	8·7	4·0	3·01	9·7
1937					
Black Diamond ..	2·54	10·6	6·5	2·90	12·3
Himalayan Giant ..	2·53	10·3	7·4	2·92	12·1
1938					
Black Diamond ..	1·74	9·7	5·6	2·95	19·7
Himalayan Giant ..	2·00	9·0	5·6	2·93	17·3

Very little difference was noted in the chemical composition of the *Loganberry* and *Phenomenal Berry*. In canning, the latter could be distinguished by its paler colour. The acidity and *p*H of loganberries does not alter appreciably throughout the season, but the total sugar-content appears to increase. No difference in chemical composition or canning properties of the two varieties of blackberries could be detected. Ripening changes in cultivated blackberries are considerable; in particular, the *p*H changed from 2·7 to 3·4 during the ripening of the fruit in the edible state.

MINERAL-CONTENT OF CANNED VEGETABLES.—In this investigation (by G. Horner) the results indicate that the losses of mineral constituents in the canning

of vegetables are of approximately the same magnitude as those that occur in cooking in the home. Calcium is the only element that normally shows an increase, the calcium present in the water being readily absorbed by vegetables during the blanching process; the increase is roughly proportional to the hardness of the water. Calcium is not evenly distributed between the solid and liquid portions of the contents of the can, the ratio of the percentage of calcium in the solids to that in the liquid ranging from 1·35 with fresh peas to 2·11 with carrots. A loss of magnesium is recorded in all the operations. The variations shown are partly due to changes in weight, but the amount of calcium present and the common salt in the cooking and canning liquids also have some effect on the behaviour of magnesium. Potassium salts are freely soluble, and their concentrations in the solid and liquid portions are virtually equal. A blanch of only three minutes in boiling water produces losses of 30 to 40 per cent., and the total losses on cooking and canning may be as high as 60 to 70 per cent. This suggests that the potassium salts in vegetables are distributed near the surface rather than in the interior. A loss of phosphorus occurs in all the operations; for example, with fresh peas, 12 per cent. in blanching, 41 per cent. in canning and 35 per cent. in home cooking. The concentration of phosphorus in the solid portion of canned vegetables is usually about double that in the liquid, indicating that roughly about half of the phosphorus is in a soluble form. Chlorine constituents of vegetables are freely soluble and become evenly distributed throughout the contents of the can.

THERMOPHILIC BACTERIA IN SUGAR.—It has been conclusively shown that sugar may be the direct cause of the entry of such large numbers of thermophilic bacteria into the cans that sterility is not attained and spoilage may ensue. When such sugar is used, scrupulous attention to cleanliness of the plant cannot eliminate the risk. In the present investigation (by T. G. Gillespy) previous work on the subject is reviewed and the results of experiments are described. It was found that many samples of sugar refined in Britain were unsuitable for canning. When unsuitable sugars were used in canning experiments with peas, sterility was not attained by normal processing. Bacteriological analyses of samples taken during sugar refining show the number of aerobes and anaerobes present at various stages. It is probable that there will be an increasing demand for guaranteed canners' sugar in this country.

COMPOSITION AND TEXTURE OF DRIED PEAS.—This investigation has been continued by W. B. Adam (*cf.* ANALYST, 1937, **62**, 738; 1938, **63**, 823). It was found that there was little difference in the texture of peas grown from seed planted at periods from March to May. Artificial drying of shelled mature peas for six to seven hours at 120° F. and 160° F. appeared to toughen the texture very slightly.

The following wide variations were noted in the percentages of the various mineral constituents in the ash: CaO, 1·8 to 5·0; MgO, 5·2 to 8·3; K_2O, 34 to 39; P_2O_5, 22 to 42. The effect of varying the temperature and time of ashing was studied. The sample of peas used was stored under conditions of uniform temperature and humidity, and in each test 20 g. were weighed into silica dishes and ignited in a muffle-furnace until the last traces of carbon had disappeared. The temperatures given in the following table are only approximate and were determined by means of thermo-couples and also by fusion of various pure salts placed near the silica dishes.

Conditions of ashing			Composition of ash			
Temp. °C.	Time Hours	Ash Per Cent.	CaO Per Cent.	MgO Per Cent.	P_2O_5 Per Cent.	K_2O Per Cent.
500	24	2·86	2·34	5·66	39·8	43·6
630	18	2·83	2·52	5·69	40·0	42·9
750	8	2·78	2·44	5·94	40·6	42·3
900	2	2·68	2·60	6·15	40·3	41·6

In another set of tests peas were ashed for various times at each of the four temperatures used before and the following results were obtained:

Conditions of ashing		
Temp. °C.	Time Hours	Ash Per Cent.
500	24	2·86
	36	2·80
	38	2·80
630	18	2·96
	27	2·87
	36	2·84
750	8	2·76
	12	2·74
	16	2·74
900	2	2·78
	3	2·71
	4	2·66

These data indicate that the greatest risk of variable results appears to be due to incomplete ashing rather than to excessive heating, although ashing at a high temperature resulted in a slight loss of potassium. Ashing in powdered form was found to be less satisfactory than ashing the whole peas.

STUDIES ON THE MOULD *BYSSOCHLAMYS FULVA*.—In continuation of his investigation (*cf.* ANALYST, 1938, **63**, 824), T. G. Gillespy has confirmed the finding that the asci of *B. fulva* are most resistant to heat at *p*H 5. The protective effect of sucrose in solutions has been clearly shown; nearly 10 per cent. of the asci survived for 12 minutes at 90° C. when heated in fruit syrups. Ascospores were found to be susceptible to intermittent heating. The optimum conditions for the shortest intermediate heating period were as follows:—Suspending medium *p*H 3 (or lower); initial and final heatings, 10 minutes at 77° C.; intermediate temperature, 46° C. All ascospores were destroyed when the intermediate period was 30 minutes. When heated at 77° C., without a break, 38 per cent. survived.

Metropolitan Water Board

THIRTY-SECOND ANNUAL REPORT ON THE RESULTS OF THE CHEMICAL AND BACTERIOLOGICAL EXAMINATION OF THE LONDON WATERS FOR THE TWELVE MONTHS ENDED 31ST DECEMBER, 1937*

OWING to the unfortunate illness of Colonel Harold early in May last year and its tragic termination in July, the completion of his report by the Deputy Director of Water Examinations, Mr. Denison B. Byles, was delayed. Tribute is paid in the preface by his deputy to Colonel Harold and to his outstanding work for the Board during his four years' service.

The Report consists of an introduction and seventeen sections under the following headings: (1) Bacteriological Section; (2) Biological Section; (3) Chemical Section; (4) Works Efficiency; (5) Walton Works; (6) Kempton Park Works; (7) Stoke Newington Works; (8) Wells; (9) Prefiltration Waters; (10) Resistance to Filtration and Microscopical appearances of Prefiltration Waters; (11) Epping Sewage and Cobbin's Brook; (12) Waltham Abbey Well; (13) Complaints; (14) The

* By Lt.-Col. C. H. H. Harold, O.B.E., M.D., Ch.B., D.P.H. 1939. Published by P. S. King & Son, Ltd., 14, Great Smith Street, Westminster. Price 10s. 6d.

Thames as a source of Potable Water; (15) Meteorological Notes; (16) Routine Tables; (17) The Flow and Bacteriology of Underground Water in the Lee Valley.

This Report, in common with previous Reports of Colonel Harold and his predecessor, contains matter of very considerable interest to all who are engaged in water examinations and undertakings, as well as particulars about the London supply. A brief account of the main points of general interest only is given in this summary.

Section I: The Bacteriological Section

Comparison of the Efficiency of Enrichment Media for the Primary Isolation of Coliform Bacilli.—The enrichment media tested were: MacConkey's broth, Brilliant Green Bile broth and Dominick–Lauter's Methylene blue-bromcresol-purple broth. These were tested on 7277 samples, and it was found that the results obtained were, on the whole, very similar with all three. Brilliant Green Bile broth yielded 786 positives of which 585 were typicals, MacConkey broth yielded 762 positives with 563 typicals, and Dominick–Lauter broth 744 positives with 543 typicals. MacConkey's broth is judged to be in no way inferior to the other two and superior to them for stored waters.

The Indole Presumptive Test.—This test, which is applied to filtered waters, consists of cultivation in MacConkey's broth and in peptone water for 18 to 24 hours; samples showing gas in the former and indole in the latter are reported at once to the engineers as unsatisfactory. A certain number of indole productions, however, are not due to *B. coli*, thus unduly weighting the number of reported samples and leading to the inference that more *B. coli* are present than there actually are.

It was decided to substitute a tube of Brilliant Green Bile broth for peptone water, and to observe the production of gas instead of indole. The value of being able to report unsatisfactory filtration so promptly—within 18 to 24 hours—that a filter can be put out of commission immediately is obvious.

Useful Modification of Koser's Citrate Medium.—Growth in this medium, indicated by turbidity, is one of the tests to distinguish *intermediate aerogenes-cloacae* types from *B. coli type I*. As an aid to the reading of turbidity it was found that addition of bromothymol blue and adjustment to *p*H 6·4 to 6·6 gave a colour-change from apple-green to blue-green or deep blue with citrate-positive cultures, some of the citrate being converted into bicarbonate and carbonate. This useful device might well be adopted in other laboratories.

Application of the MacConkey and Koser's Citrate Tests to the Quantitative Estimation of *Coli* and *Aerogenes* Organisms in Raw Water.—A comparison is made of three methods: (1) The customary routine method of the Board. (2) Method (*a*) [modification* of Method III of Wilson†]. Two sets of MacConkey's broth cultures are prepared, and one is incubated at 42° C., and the other at 44° C. for 24 hours. Tubes showing acid and gas at 42° C. are subcultured into citrate broth and incubated at 37° C. for 48 hours. Acid and gas at 44° C. indicate *B. coli type I*, and turbidity in citrate broth at 37° C. indicates intermediate-aerogenes-cloacae types. (3) Method (*b*) [modification* of Method IV of Wilson]—one set of MacConkey's broth cultures is incubated at 42° C. and, after 24 hours, all positives are subcultivated into MacConkey's broth and incubated at 44° C., and into citrate broth and incubated at 37° C. The rationale is the same as for method (*a*), except that delicate organisms that might, and do, fail to grow at 44° C. grow after preliminary cultivation at 42° C., and that the same inoculum of water is used, and *B. coli type I* and intermediate-aerogenes organisms are thus given an equal chance.

* The modification consists only in incubating at 42° C. instead of 37° C.
† Vide *The Bacteriological Grading of Milk.*

The enumeration of Coliform bacilli by methods (*a*) and (*b*) was checked by subsequent plating out, isolation and identification of types. Method (*a*) was found to inhibit the growth of many typicals; method (*b*) did so only in one instance. The latter method was therefore selected for further trials with 56 samples of raw water. It was found that by method (*b*), which merely involves presumptive selective media and temperatures and takes only 72 hours, 46 out of 56 samples were correctly analysed as regards their *Coli-aerogenes* contents, 96 hours being required by the routine tests. While, therefore, it is not as good as the routine test, in view of its far greater simplicity it should be of considerable value where laboratory facilities are limited, and where time is an important consideration.

Section II.—The Biological Section

The outstanding investigations under this heading were: (1) the study of algal flora in standing storage reservoirs and the conditions which lead to a fall in the nitrate nitrogen content and the development of blue-green algae, such as *Oscillatoria*, and (2) the increase in volume of sand grains in primary filters by deposition of calcium carbonate, leading to less efficient filtration. It is shown that the deposition of calcium carbonate is not due to removal of carbon dioxide and consequent alteration of the carbonate-bicarbonate equilibrium, and that it is more probably due to biological agencies. Certain suggestions are made as to possible remedial measures.

Section XVI.—Routine Tables

These show absence of typical *B. coli* in 100 ml., in 99·6 per cent. of all samples of Thames-derived waters examined (3163); in 99·0 per cent. of all London waters as supplied to consumers (7168 samples); in 98·4 per cent. of all samples from the Kent wells; in 96·9 per cent. of samples from Lee Valley wells—a very high standard of purity.

Section XVII.—The Flow and Bacteriology of Underground Water in the Lee Valley

Previous tests with fluorescein proved that there is direct fissure communication between the swallow holes at North Mimms and South Mimms and Chadwell Spring, Amwell Marsh Well and Hoddeston Well, and that water travels from these swallow holes to the spring and wells—a distance of some 10 miles—in 3 days. Such a rate provides little possibility of purification. Bacteriological examinations were made of the impure water flowing into the swallow holes and of the water from the spring and wells, and the frequence and distribution of the *Coli-aerogenes* types present at the sampling points was worked out. Generally speaking, it was found that reduction of pollution of the water from the Lee Valley was two hundredfold in drought, but in flood times only tenfold. The relative numbers of *Coli-aerogenes* bacteria were determined by direct plating, so that the original proportions should not be modified by preliminary cultivation in enrichment media.

It was found that from all sampling points *B. coli type I* accounted for 74 per cent. *intermediate type I* for 9·6 per cent., *B. coli type II* for 5·4 per cent., *aerogenes-cloacae types* for 4·1 per cent., *intermediate type II* for 3·8 per cent., and *irregular strains* for 3·0 per cent., and that with minor differences these proportions held whether the samples were from the swallow holes or from the spring and wells. It is concluded that when assessing the purity of samples in this country, the intermediate and aerogenes types, representing some 25 per cent., cannot be ignored. It is probable, however, though the report gives no data on this point, that the number of samples of this contaminated water failing to show the presence of *B. coli type I* in one or other of the quantities, taken in ordinary routine and with the use of enrichment media examination, would be quite small. D. R. Wood

British Pharmacopoeia Commission

REPORTS OF COMMITTEES ON PHARMACEUTICAL CHEMISTRY*

WE are asked to state that these Reports include recommendations relating to the monographs of the British Pharmacopoeia, 1932, as amended by the Addendum, 1936. They also include draft monographs on new substances which the Commission have provisionally accepted for inclusion in the next Pharmacopoeia. In accordance with former practice these Reports are made public, in order to provide an opportunity for medical practitioners, pharmacists, analysts, manufacturers and others who may be interested, to criticise them and to suggest emendations before the preparation of the new Pharmacopoeia reaches its final stage.

REPORT No. 11

I. REPORT OF THE SUB-COMMITTEE ON ESSENTIAL OILS.—The Sub-Committee (Messrs. T. Tickle, *Chairman*, C. T. Bennett, S. W. Bradley, T. T. Cocking, C. E. Sage, and W. H. Simmons) recommend that the following monographs and appendixes of the B.P. 1932, as amended by the Addendum, 1936, should be continued without change:—Oleum Abietis, Oleum Anethi, Oleum Anisi, Oleum Cajuputi, Oleum Cari, Oleum Eucalypti, Oleum Lavandulae, Oleum Menthae Piperitae, Oleum Santali Australiensis.

Appendix XI. B. The Determination of Free Alcohols in Volatile Oils.

Appendix XI. C. The Determination of Aldehydes in Volatile Oils.

Appendix XI. D. The Determination of Carvone in Oil of Caraway, and in Oil of Dill.

Appendix XI. E. The Determination of Cineole in Oil of Cajuput and in Oil of Eucalyptus.

Changes or additional requirements are recommended in connection with: Eucalyptol, Oleum Cadinum, Oleum Caryophylli, Oleum Chenopodii, Oleum Cinnamomi, Oleum Coriandri, Oleum Limonis, Oleum Myristicae, Oleum Rosmarini, Oleum Santali, Oleum Terebinthinae.

Appendix XI. A. (Determination of the Esters in Volatile Oils.) Changes are proposed to bring the method into agreement with the Society of Public Analysts' method.

Appendix XI. E. (Determination of Cineole.) The present Appendix should be retained, with the omission of the reference to Eucalyptol and the addition of the direction that the *o*-cresol is to be accurately weighed.

The following new monographs are proposed:—Oleum Amygdalae Volatile Purificatum, Oleum Camphorae Rectificatum, Appendix XI. C. [3] (Purified Volatile Oil of Bitter Almond), Determination of Volatile Oil in Crude Drugs (a modification of the apparatus and method of Cocking and Middleton is recommended and described).

II. REPORT OF THE SUB-COMMITTEE ON FIXED OILS, FATS, WAXES, RESINS AND SOAPS.—The Sub-Committee (Messrs. T. Tickle, *Chairman*, E. R. Bolton,† N. Evers, J. R. Nicholls, and W. H. Simmons) recommended that the following monographs of the B.P. 1932, as amended by the Addendum, 1936, should be continued without change:

Cera Alba, Cera Flava, Oleum Amygdalae, Oleum Hydnocarpi, Oleum Hydnocarpi Aethylicum, Paraffinum Molle Album, Paraffinum Molle Flavum, Sevum, Appendix IX. A. (Test for Absence of Cottonseed Oil in Other Oils), Appendix IX. B. (Test for the Absence of Sesame Oil in Other Oils), Appendix X. B. (Determination of the Saponification Value of Fixed Oils and Fats).

It is recommended that changes or additional requirements should be inserted for:

Oleum Arachis, Oleum Gossypii Seminis, Oleum Lini, Oleum Olivae (Fitelson's colour test for Tea-seed Oil recommended), Oleum Ricini, Oleum Sesami, Oleum Theobromatis, Paraffinum Durum, Paraffinum Liquidum, Sapo Animalis (tests redrafted in accordance with new methods published by the Society of Public Analysts), Sapo Durus (new S.P.A. methods), Sapo Mollis (new S.P.A. methods), Appendix IX. C.—Test for Absence of Arachis Oil in Other Oils (new directions proposed), Appendix X. A.—Determination of the Acid Value of Fixed Oils, Fats and Resins, Appendix X. C.—Determination of Iodine Value of Fixed Oils and Fats (Pyridine bromide method recommended), Appendix X. D.—Determination of Unsaponifiable Matter in Fixed Oils and Fats (S.P.A. method recommended), Oleum Hippoglossi. (It is recommended *inter alia* that when a statement is made of the number of units of vitamin D in Halibut Liver Oil, the units enumerated should be the units described under the biological assay of antirachitic vitamin [vitamin D].)

III. REPORT OF THE SUB-COMMITTEE ON THE ASSAY OF GALENICALS.—The Sub-Committee (Messrs. T. Tickle, *Chairman*, T. T. Cocking, C. E. Corfield, J. Evans, N. Evers, D. C. Garratt,

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* Reports Nos. 11 and 12. May, 1939. Published by Authority of the General Medical Council, 44, Hallam Street, London, W.1. Price 1s. 6d. each.

† Deceased February, 1939.

W. H. Linnell, J. R. Nicholls and A. D. Powell) recommend that the following assays should be continued without change:

Extractum Filicis, Linimentum Camphorae, Liquor Ammonii Acetatis Dilutus, Liquor Ammonii Acetatis Fortis, Liquor Arsenicalis, Liquor Arseni et Hydrargyri Iodidi, Liquor Calcii Hydroxidi, Liquor Formaldehydi, Liquor Glycerylis Trinitratis, Liquor Iodi Fortis, Liquor Iodi Mitis, Liquor Iodi Simplex, Liquor Magnesii Bicarbonatis, Liquor Potassii Hydroxidi, Liquor Quininae Ammoniatus, Liquor Sodii Chloridi Chirurgicalis, Oxymel, Phenol Liquefactum, Pilula Hydrargyri, Spiritus Aetheris Nitrosi, Syrupus Ferri Iodidi, Unguentum Hydrargyri Dilutum, Unguentum Hydrargyri Compositum, Appendix XII. A. (Determination of Ash), Appendix XII. B. (Determination of Acid-insoluble Ash), Appendix XII. C. (Determination of Water-soluble Ash), Appendix XII. D. (Determination of Alcohol-soluble Extractive), Appendix XII. E. (Determination of Water-soluble Extractive), Appendix XII. G. (Determination of Industrial Methylated Spirit).

It is recommended that changes or additional requirements be inserted for the following:

Extractum Malti (limit test of diastatic value corresponding approximately to 15° Lintner), Extractum Malti cum Oleo Morrhuae, Ferri Carbonas Saccharatus, Glycerinum Acidi Borici, Glycerinum Acidi Tannici, Glycerinum Boracis, Glycerinum Phenolis, Jalapa, Linimentum Belladonnae, Linimentum Saponis, Linimentum Camphorae, Linimentum Camphorae Ammoniatum, Linimentum Terebinthinae, Linimentum Terebinthinae Aceticum, Liquor Cresolis Saponatus, Liquor Ferri Perchloridi (lead limit of 15 p.p.m. proposed), Liquor Hydrargyri Perchloridi, Liquor Plumbi Subacetatis Fortis, Oxymel Scillae, Pilula Ferri Carbonatis, Spiritus Ammoniae Aromaticus, Spiritus Camphorae, Syrupus Ferri Phosphatis Compositus, Tabella Glycerylis Trinitratis (assay methods still under investigation), Unguentum Acidi Borici, Unguentum Acidi Salicylici, Unguentum Acidi Tannici (no suitable method yet available), Unguentum Hydrargyri Ammoniati, Unguentum Hydrargyri Oleati, Unguentum Hydrargyri Subchloridi, Unguentum Phenolis, Unguentum Sulphuris, Unguentum Zinci Oleatis, Unguentum Zinci Oxidi.

In Appendix XII. F.—Determination of Alcohol Content—Method II has been revised. In the *Alcohol Limits Table* the following changes should be made.

	Methods of determining alcohol content	Alcohol limits per cent. v/v of ethyl alcohol
Extractum Ipecacuanhae Liquidum ..	III	75–80
Infusum Aurantii Concentratum	III	21–25
Infusum Buchu Concentratum	III	21–25
Infusum Caryophylli Concentratum ..	III	22–25
Spiritus Aetheris Nitrosi	II	84–87
Tinctura Capsici	III	57–60
Tinctura Catechu	I	37–40
Tinctura Cinchonae	I	63–66
Tinctura Ipecacuanhae	III	20–24
Tinctura Opii Camphorata	II	56–60
Tinctura Rhei Composita	III	48–53
Tinctura Senegae	I*	55–58
Tinctura Zingiberis Fortis	III	80–88

REPORT No. 12

I. Report of the Sub-Committee on Synthetic Chemicals.—The Sub-Committee (Dr. W. H. Linnell, *Chairman*, Messrs. J. W. Blagden, F. H. Carr, A. J. Ewins, H. King, A. D. Powell, and S. Smith) recommend that changes or additional requirements be inserted for:

Acriflavina, Adrenalina, Barbitonum, Barbitonum Solubile, Calcii Gluconas, Carbromalum, Chloralis Hydras, Cinchophenum, Dextrosum, Fluoresceinum Solubile, Indicarminum, Iodophthaleinum, Lactosum, Methylthioninae Chloridum, Phenobarbitonum, Phenobarbitonum Solubile, Salicinum, Thymol.

New Monographs.—Acidum Mandelicum (Phenylglycollic Acid), Calcii Laevulas, Calcii Mandelas, Cyclopropanum, Liquor Nucleotidi, β-Phenylisopropylamine, β-Phenylisopropylamine Sulphate, Pyridine-β-Carboxylic Acid Diethylamide, Sulphanilamidum, Theophyllina cum Aethylenediamine, Urethanum.

II. Report of the Sub-Committee on Alkaloidal Assays.—The Sub-Committee (Dr. W. H. Linnell, *Chairman*, Messrs. N. L. Allport, C. E. Corfield, N. Evers, D. C. Garratt, A. D. Powell, and L. W. Ragg) recommend that changes or additions should be made for the following:

Aconitum, Extractum Ipecacuanhae Liquidum, Ipecacuanha Pulverata, Liquor Morphinae Hydrochloridi, Liquor Strychninae Hydrochloridi, Lobelia (Society of Public Analysts' method),

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* Acidify with *dilute sulphuric acid* before the first distillation.

Opium, Pulvis Ipecacuanhae et Opii, Syrupus Ferri Phosphatis cum Quinina et Strychnina, Tinctura Opii Camphorata, Theobromina et Sodii Salicylas, Theophyllina et Sodii Acetas (under investigation).

Alkaloidal Salts.—The Sub-Committee have agreed to accept as a general principle that for any alkaloidal salt, the purity of which can be accurately controlled by physical measurements and by chemical tests for purity, a chemical assay is unnecessary. It is recommended that no assay should be prescribed for: Apomorphinae Hydrochloridum, Atropinae Sulphas, Cocainae Hydrochloridum, Ephedrinae Hydrochloridum, Ergotoxinae Aethanosulphonas, Hyoscinae Hydrobromidum, Physostigminae Salicylas, Pilocarpinae Nitras. Changes in the assay of a number of alkaloidal salts are recommended, and a new monograph for Morphinae Sulphas is proposed.

British Standards Institution

THE following Standard Specifications have been issued:*

NO. 844—1939. BRITISH STANDARD METHODS FOR THE TESTING OF VEGETABLE ADHESIVES.

These methods are intended to apply to such vegetable adhesives as are manufactured from starch or from material containing a high proportion of starch, *e.g.* wheat flour and potato starch. Typical adhesives are crystal gums, dextrins and water-soluble pastes and powders.

The physical and chemical properties of vegetable adhesives depend, first, on the nature of the raw materials, and secondly, on the methods of processing. It is not possible to draw up any simple test that will evaluate completely the quality of an adhesive, or its suitability for a particular trade. The tests included, however, provide indications of the behaviour of the adhesive in use, and may be taken as reliable criteria of the quality of different deliveries.

The tests described comprise methods for determining moisture-content, acidity, alkalinity and *p*H value, ash, chemical residues, colour, efficiency of adhesion, foam, keeping quality and viscosity. Some of the physical tests are of an empirical nature (*e.g.* the foam test), but if the Methods are closely followed, they will give consistent results which will afford useful information both to the manufacturer and to the user. Some work has been done on Tackiness, Rate of Setting, and Viscosity at machine consistencies, but the tests have not yet been sufficiently developed to admit of standardisation.

Where possible, the tests are divided into two classes: A.—*Approximate Tests*, which have been found satisfactory for routine testing. B.—*Standard Tests*, which, in the hands of skilled workers, give more precise and reliable results than the approximate tests.

The following directions, *inter alia*, are given for the tests:

Moisture-content is defined as the percentage loss in weight of the sample as received, when the adhesive is dried at 105°–110° C. to constant weight. Liquids and pastes are first mixed with recently ignited silver sand.

Alkalinity, Acidity and pH.—Fifty ml. of a solution containing 10 g. of adhesive in 250 ml. of water are titrated with 0·1 *N* hydrochloric acid, with phenolphthalein as indicator. The alkalinity is calculated as the percentage of sodium hydroxide on the moisture-free weight. If acid, the sample is titrated with 0·1 *N* sodium hydroxide solution, with methyl orange as indicator, and the acidity is expressed as the percentage of hydrochloric acid on the moisture-free weight.

To determine the *p*H value 5 g. of the sample are dispersed in 100 ml. of freshly-boiled distilled water, and the *p*H is determined by a standard electrometric method. A complete titration curve is prepared by plotting the *p*H values, obtained after successive small known additions of 0·1 *N* hydrochloric acid (or 0·1 *N* sodium hydroxide solution), against the amounts of acid or alkali added.

Ash.—Five g. are incinerated in a platinum basin over a low flame at a dull red heat (not exceeding 500°–550° C.).

Chlorides.—The ash is extracted with hot distilled water and filtered, and the filtrate is neutralised with acetic acid (or ammonia), treated with 0·1 ml. of 10 per cent. acetic acid, and titrated with standard silver nitrate solution (1 ml. ≡ 0·01 g. of sodium chloride), 1 ml. of 10 per cent. potassium chromate solution being used as indicator. To avoid the possibility of loss of chlorides by volatilisation, the adhesive (in solution) may be dried and ignited with an equal weight of chloride-free lime, the residue extracted with boiling water, and the chloride determined in the filtrate.

Reducible Sulphur.—The adhesive is treated in a special flask (described and illustrated) with 10 ml. of pure sulphur-free phosphoric acid and 1 g. of pure (not activated) zinc, the flask is heated on a steam-bath for one hour, so that the gases evolved pass through filter-paper impregnated

* Obtainable from the Publication Department, British Standards Institution, 28, Victoria Street, London, S.W.1. Price 2s. net, post free 2s. 2d. each.

with a 10 per cent. w/v solution of lead acetate, and the sulphide stain is compared with standard stains produced in a similar manner.

Colour.—The colour of the adhesive is compared with that of an agreed standard adhesive, the samples being spread in thin films of equal thickness on glass and allowed to dry at a temperature not higher than 50° C. If there is no agreed standard adhesive, the colour shall be compared with agreed standard coloured strips. In cases of dispute the colour shall be measured by a standard photometric method.

Efficiency of Adhesion.—A uniform film of the adhesive is applied by means of a brush to a strip (1 inch wide) of one of the materials, and immediately afterwards a similar strip of the second material is applied to the glued place and firmly pressed down by hand. The strips are left for the times specified in the following table, after which the joint strength is assessed by attempting to separate the two strips by peeling. If they can be separated only by continuous disruption of the surfaces (however slowly the peeling is carried out), the efficiency of adhesion is considered satisfactory. In the Standard Test the materials are kept flat until dry by placing them (under specified conditions) under a load of 1 kg., and standard conditions of humidity (34 and 66 per cent.) are maintained.

Class of material	Drying time	Time under specified load
Paper and cardboard	Not less than 6 hours	½ hour
Transparent cellulose material	„ „ „ 1 week	24 hours
Paper and cardboard with a finish or lining of wax, varnish lacquer or metallic foil ..	„ „ „ 1 „	24 „

Maintaining Standard Humidity.—A humidity of 34 per cent. is obtained over magnesium chloride crystals at temperatures between 18° and 24° C., and 66 per cent. humidity is obtained over a saturated solution of sodium nitrite at temperatures between 18° and 24° C.

Foam.—Five g. of the adhesive are dispersed in 50 ml. of water, and the mixture is left for 30 minutes in a graduated stoppered 100-ml. cylinder (B.S. 604) at 25° C. ± 0·2° C. The cylinder is then shaken vigorously with a throw of about 12 in., at a rate of three shakes per second, for one minute, after which the time required for the height of the liquor to correspond to 45 ml., and also the volume of the foam above the liquid, are recorded.

Keeping Quality.—Five g. of adhesive are dispersed in 20 ml. of sterile distilled water and transferred to a Petri dish (B.S. 611), which is covered and incubated at 40° C. Every 24 hours the Petri dish is removed from the incubator and allowed to cool before inspection, and the periods when putrefaction or moulds (or both) occur are noted.

Viscosity.—Owing to the lack of a suitable method for such viscous materials, the method provisionally recommended is the determination of the dilution necessary to obtain a definite viscosity. The dilution of a dispersion of 100 g. of adhesive in distilled water at 20° C. shall be such that the viscosity is 500 centistokes (CS), when determined by the method described in Clause 5 of B.S. 188.

No. 849—1939. British Standard Specification for Burettes and Bulb Burettes.

Burettes.—The series includes the following sizes:

Total capacity		1 ml. sub-divided into		0·01 ml. intervals.
„	„	2 „ „ „	„	0·02 „ „
„	„	5 „ „ „	„	0·02 „ „
„	„	5 „ „ „	„	0·05 „ „
„	„	10 „ „ „	„	0·02 „ „
„	„	10 „ „ „	„	0·05 „ „
„	„	10 „ „ „	„	0·1 „ „
„	„	25 „ „ „	„	0·05 „ „
„	„	25 „ „ „	„	0·1 „ „
„	„	50 „ „ „	„	0·1 „ „
„	„	100 „ „ „	„	0·2 „ „

Bulb Burettes.—The series includes the following sizes:

Total capacity ml.	Bulb capacity ml.	Range and sub-division of scale ml.
45	20	20 to 45 in 0·05 ml. intervals
65	40	40 „ 65 „ 0·05 „ „
85	60	60 „ 85 „ 0·05 „ „
105	80	80 „ 105 „ 0·05 „ „

For each series definitions of capacity and tolerances on capacity are given, and there are specifications for the material, dimensions, finish of top, stopcocks, jet, delivery time, graduation marks and (for the bulb burettes) shape of bulb.

Draft Revised British Standard No. 410, for Test Sieves.

A limited number of copies of this Draft Specification are available for purposes of technical comment. Applications for copies, and comments thereon, should be addressed to the British Standards Institution.

BRITISH STANDARDS FOR A.R.P. REQUIREMENTS

The Home Office Air Raid Precautions Department has arranged with the British Standards Institution to prepare and issue on their behalf a special A.R.P. series of British Standards for Air Raid Precaution purposes.

The preparation of these Standards will be under the control of a special Advisory Committee and, in accordance with the usual practice of the B.S.I., will be carried out in the closest co-operation with Government Departments and the industry concerned.

All communications relating to this work should be addressed to the British Standards Institution, 28, Victoria Street, Westminster, S.W.1.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

Alcohols as a Measure of Spoilage in Canned Fish. D. A. Holaday. (*J. Assoc. Off. Agr. Chem.*, 1939, **22**, 418–420.)—During a study of spoilage in canned fish it appeared that a determination of the amount of alcohols present would afford a reliable index of decomposition. The method used for the determination of alcohols was essentially that of Friedmann and Klaas (*J. Biol. Chem.*, 1936, **115**, 47), depending upon controlled oxidation by alkaline potassium permanganate solution following the removal of interfering substances, and which is specific for the alcohols as a group. Since more than one alcohol may participate in the reaction, the results are expressed as ml. of 0·02 *N* potassium permanganate solution per 100 g. of sample. Fresh samples contain a small amount of alcohols, and this amount increases progressively as decomposition proceeds. The results found with 3 samples ranging in quality from fresh to decomposed were as follows: mackerel, 3·2 to 4·2, 8·6 to 18·0, 27·6 to 70·0; salmon, 3·7 to 7·1, 6·5 to 17·2, 25·2 to 62·2; sardines, 3·3 to 10·2, 11·6 to 27·3, 65·8 to 117·6. To identify the alcohols present the distillate (2·6 litres) from 360 g. of putrid fish was purified as described (*loc. cit.*) and concentrated by a system of fractional distillation to a volume of 8 ml. From 2 ml. of this distillate fractionation in a micro-apparatus yielded a concentrate of 0·05 ml. This had a strong odour of ethyl alcohol, and the m.p. of its 3:5-dinitrobenzoate proved it to consist mainly of this alcohol. The procedure for the determination of the amount of alcohols was as follows:—The entire contents of a can were passed through a meat chopper three times. A portion of the minced product (50 g.) was made into a uniform suspension with water (100 ml.) and treated with 15 ml. of 2 *N* sulphuric acid and 15 ml. of 20 per cent. phosphotungstic acid, and, after dilution to 250 ml., the mixture was filtered. The filtrate (150 ml.) was mixed with 10 ml. of Denigès' reagent (U.S.P. XI), made up to 200 ml. and distilled in an all-glass apparatus until 100 ml. of distillate

had been collected. The distillate was treated with 5 ml. of Denigès' reagent and with a suspension of lime (100 g. of calcium oxide in 1 litre of water) until the mixture became orange. After dilution to 150 ml. the mixture was distilled and 100 ml. of the distillate were collected. An aliquot portion of the distillate was treated with 10 ml. of 5 *N* sodium hydroxide solution and 25 ml. of 0·02 *N* potassium permanganate solution and heated on the water-bath for at least 20 minutes. When cold, the liquid was acidified with 10 ml. of *N* sulphuric acid, and sufficient potassium iodide was added to react with the unaltered permanganate. Finally, the liberated iodine was titrated with 0·02 *N* sodium thiosulphate solution. Each ml. of 0·02 *N* potassium permanganate solution is equivalent to 0·0855 mg. of ethyl alcohol. The aliquot portion of the distillate should be such that not more than 6 ml. of potassium permanganate solution are required. Usually 25 ml. is a suitable aliquot portion, but if a smaller aliquot portion is taken, the volume in the reduction flask should be adjusted with water to 60 to 70 ml. The reaction is not linear, but gives consistent results if the conditions of time, volume and concentration of alkali are as stated. A. O. J.

Volatile Fatty Acids as an Indication of Spoilage in Canned Sardines. F. Hillig. (*J. Assoc. Off. Agr. Chem.*, 1939, **22**, 414–418.)—Previous work (*J. Assoc. Off. Agr. Chem.*, 1938, **21**, 684; 1938, **21**, 688; Abst., ANALYST, 1939, **64**, 44; 1939, **22**, 116) on the determination of volatile fatty acids in canned salmon, tuna fish and herring roe is now extended to include canned sardines, the volatile fatty acid number and formic acid number being determined by the method previously described (Abst., ANALYST, *loc. cit.*). When prepared from the freshest materials, canned sardines contain a small amount of volatile acids, and this amount increases progressively as decomposition proceeds. Four samples prepared from authentic material and representing stages of spoilage from fresh to badly decomposed gave the following figures:—formic acid number, 1·2 to 1·8, 1·5 to 1·8, 3·3 to 6·0 and 15·5 to 21·1; volatile fatty acid number, 15·8 to 18·6, 21·6 to 24·5, 57·1 to 80·8, and 156·1 to 201·5. With fish packed in sauce (especially in mustard preparations) the volatile fatty acid number has only a limited value as a criterion of freshness, but the formic acid number still serves as a valuable index. The formic acid number of sardines canned in a mustard preparation (1·7 to 2·0) did not differ greatly from that of the same fish packed without sauce (1·5 to 1·8), but when the fish were packed in tomato sauce the number was slightly higher (2·1 to 3·2). Attempts were made to identify the individual acids present. From a study of the fractional distillation curves it was concluded that the highest detectable member of the series present was propionic acid with possibly traces of one or more higher members of the series. A. O. J.

Grading Milk by the Resazurin Test. J. G. Davis. (*Food Manufacture*, 1939, **14**, 196–198, 207.)—Resazurin is a blue dyestuff which is prepared by the interaction of resorcinol and nitric acid containing nitrous acid. It is an oxidation-reduction indicator (range, +0·18 to +0·09 volt), and on reduction it is first converted irreversibly into the pink resorufin and then, reversibly, into the colourless di-hydroresorufin. Both resazurin and resorufin are indicators of

acidity, the former being blue at *p*H 6·5 and red at *p*H 5·3, and the latter pink at *p*H 6·5 and yellow at *p*H 4·8. Since the oxidation-reduction range of methylene blue is +0·12 to +0·03 volt, and the normal redox-potential of fresh milk is just over +0·2 volt, it is evident that very weak reducing systems in milk, irrespective of their origin, will affect resazurin much more than methylene blue. Although as a consequence resazurin is reduced more rapidly, and therefore enables a result to be obtained more quickly than with methylene blue, it is sensitive to reducing systems other than those that reduce methylene blue in milk (*cf.* Hobbs, *J. Dairy Res.*, 1939, **10**, 35); examples of the latter are the effects of bacteria such as *Coli aerogenes*, lactic acid streptococci and staphylococci, which in the logarithmic phase of growth may rapidly reduce the redox-potential of milk from +0·2 to less than −0·2 volt. Although non-bacterial factors (*e.g.* reducing enzymic systems) may influence the rate of reduction of methylene blue in milk, the actual reduction itself is invariably due to bacterial development. A fair degree of correlation exists in fact between the bacterial-content of milk and the rate of reduction of methylene blue, because, although only some of the bacteria present initially are responsible for the final reduction, on an average these are a reasonably constant proportion of the entire flora. In special circumstances, however, and particularly when high cell-counts (*e.g.* due to mastitis) are obtained, almost complete reduction of methylene blue by a fresh sample of the milk may occur immediately, although the colour may return after about 30 minutes, after which true reduction occurs (*cf.* McClemont and Davis, *id.*, 1939, **10**, 88). Nevertheless, as a test for bacterial count resazurin is inferior to methylene blue; it has a greater susceptibility to the weakly-reducing systems associated with them, and the fact that it is more rapid in action does not compensate for the resulting loss in accuracy. The value of resazurin depends on the fact that it is more sensitive to the weakly-reducing systems of the cells (leucocytes and tissue cells), and therefore it is more responsive to mastitis; thus it is likely to be of more interest to manufacturers for the control of milk-processing and especially for cheese-making (*cf.* Davis and McClemont, *id.*, 1939, **10**, 94) than to those concerned with the liquid milk market; where the bacterial count is the primary concern the methylene blue test should be used. The procedure for the test is similar to that of the methylene blue test (*cf.* Davis, *Dairy Ind.*, 1939, **3**, 214, 25; 1939, **4**, 83). The resazurin should be of good quality, as some samples on the market are of no use; the author used Eastman's product. A graduated test-tube is filled to the 10-ml. mark with the sample, under aseptic conditions, the mouth of the sample-bottle and of the test-tube being "flamed." One ml. of a 0·0005 per cent. solution of resazurin is added, and the tube is closed, inverted twice, and placed in a bath at 37° C. Resazurin is sensitive to light, and both the tubes and the stock 0·05 per cent. solution from which the reagent is prepared should be kept in the dark (the latter in a refrigerator). On reduction the colour normally changes from blue, through mauve and pink to colourless, and the results may therefore be recorded in terms of the colour after a definite period (*e.g.* 1 hour), or as the time required to produce a definite colour. The pink shade is usually sufficiently distinct for the latter purpose and the time to attain this is 75 per cent. of that required for complete decolorisation (*cf.* Johns, *Amer. J. Pub. Health*, 1939, **29**, 239); moreover,

it is this earlier stage of reduction which corresponds most closely with the effects of non-bacterial reducing systems. The advantages of the first method, however, are that it enables the test to be completed in 1 hour, and it allows compensations for changes in temperature to be made more easily. It is further facilitated by means of colour standards painted on cardboard. The scale suggested is:—(1) Blue, excellent; (2) slightly mauve, very good; (3) mauve, good; (4) pink-mauve, fair; (5) mauve-pink, poor; (6) pink and (7) colourless, bad. These assessments refer to both bacterial quality and freedom from pathological abnormality. It is recommended that three tests should be made on each sample, and that when two or all of these give results corresponding with standards, 5, 6 or 7, a direct microscope test should at once be made, *e.g.* by staining a smear of the milk with polychrome or Loeffler's methylene blue (*cf.* Barrett and others, *J. Dairy Sci.*, 1937, **20**, 705; and Jenkins, *id.*, 1938, **21**, 141). The reduction of dyestuffs in milk may be influenced by factors other than bacteria, *e.g.* the breed of the cow, the season of the year, and the fat-content; further investigations on these influences are desirable. J. G.

Detection of Glucose Syrup in Honey by the "Acetic Acid Method." **P. N. Raikow.** (*Z. anal. Chem.*, 1939, **117**, 100–102.)—The presence of glucose syrup in honey has been detected (Raikow, *Z. anal. Chem.*, 1939, **116**, 40) by the formation of a white turbidity on pouring 1 ml. of glacial acetic on to about 5 g. of the honey in a test-tube. This turbidity, which may appear immediately or may be delayed, in the upper, acetic acid layer is caused by the dextrin of the glucose syrup. It was found that on allowing the test-tube to stand for some time the turbidity gradually decreased and finally disappeared, leaving a clear liquid. Recent experiments indicate that this is due to the absorption of atmospheric moisture by the acetic acid; with honey containing 10 per cent. of glucose, acetic acid containing more than 3 per cent. of moisture forms no turbidity even after long standing. With 97 per cent. acid a dense white turbidity appears; with 98 and 99 per cent. acid the turbidity is denser and almost as great as that with anhydrous glacial acetic acid. The reaction occurs more rapidly and is more pronounced if, after addition of acetic acid, the test-tube is warmed for a short time in the boiling water-bath and then allowed to cool without shaking. As honey is only slightly miscible with glacial acetic acid and the reaction therefore occurs only at the surface of contact, 5 g. are not required and even 1 to 2 g. are sufficient for the test. One gram of a 50 per cent. solution of pure glucose syrup in water gives a white turbidity with 4·5 ml. of glacial acetic acid; this disappears on warming the mixture, but re-appears on cooling. With less acid a curdy white precipitate is formed at the surface of the solution but disappears on shaking. The following modification of the original test is now proposed:—To about 5 ml. of acetic acid in a test-tube one drop of honey is added with a glass rod, the mixture is heated, with shaking, in a boiling water-bath and then cooled. If a white turbidity appears the test can be repeated several times by the addition of further drops of honey. The turbidity becomes denser each time until the limiting solubility of the glucose syrup in acetic acid is reached, after which further addition of honey causes the acid gradually to become clear; it again becomes turbid on addition of more acetic

acid. By this modification of the test very small amounts of honey can be used, and by diminution of the acid used the very smallest amounts of glucose syrup can be detected. E. B. D.

Green Turtle Oil. C. Hata. (*J. Soc. Chem. Ind., Japan*, 1939, **42**, 88B.)—The present sample of oil from the green turtle, *Chelonia japonica* (Thunberg) was pure body oil, free from liver oil; the yield was about 1·6 per cent. The oil was reddish-orange, gave a considerable deposit at ordinary temperature, was soluble in acetone, and gave hardly any reaction in the antimony trichloride test. It had the following characteristics: sp.gr. at 30/4° C., 0·9127; n_D^{30}, 1·4649; saponification value, 210·3; iodine value, 80·08; acid value, 1·0; unsaponifiable matter (mainly cholesterol and oleyl alcohol), 1·3 per cent. The fatty acids were liquid at 32° C., and had sp.gr. at 40/4° C., 0·8788; n_D^{40}, 1·4512 neutralisation value, 213·62; iodine value 81·79. The yield of ether-insoluble bromide was 3·2 per cent. with bromine-content 66·11 per cent., and the yield of bromide insoluble in petroleum spirit was 1·0 per cent. with a bromine-content of 59·58 per cent. The following acids were identified: zoomaric, oleic, an isomer of linolenic acid, $C_{18}H_{30}O_2$, a small quantity of clupanodonic, and a new highly unsaturated acid, $C_{26}H_{44}O_2$, called chelonic acid; this had neutralisation value, 141·26; iodine value, 258·05; it yielded an insoluble bromide, m.p. 248–250° C., containing 61·75 per cent. of bromine. (*Cf. J. Soc. Chem. Ind. Japan*, 1937, **40**, 185B; Abst., ANALYST, 1937, **62**, 619; *Biochem. J.*, 1938, **32**, 681; Abst., ANALYST, 1938, **63**, 442; also ANALYST, 1935, **60**, 650). D. G. H.

Okigisu Fish Oil (*Argentina kagoshimae*, Jordan and Snyder). S. Ueno and T. Tamura. (*J. Soc. Chem. Ind., Japan*, 1939, **42**, 150–151B.)—Okigisu is a small fish widely distributed in Japanese waters. Two samples of oil, of a reddish-orange and reddish-brown colour respectively, had the following characteristics:—sp.gr. at 15/4° C., (*a*) 0·9226, (*b*) 0·9229; n_D^{20}, (*a*) 1·4760, (*b*) 1·4743; saponification value, (*a*) 186·0, (*b*) 188·8; iodine value, Wijs, (*a*) 148·3, (*b*) 150·7; acid value, (*a*) 47·8, (*b*) 9·5; unsaponifiable matter, (*b*) 2·45 per cent. The mixed fatty acids from sample (*b*) had m.p. 31·0–32·5° C., iodine value, 161·7, and neutralisation value, 191·6. Separation by the lead salt and alcohol method gave 18·5 per cent. of solid acids with m.p. 51·3–52·2° C.; iodine value, 8·9; neutralisation value, 213·2; liquid acids, 81·5 per cent., with iodine value 194·8 and neutralisation value, 188·1. About 580 g. of solid acids were methylated and fractionally distilled, and an examination of the fractions revealed the presence of myristic, palmitic, stearic, behenic and tetracosanic (probably *n*-tetracosanic) acids, with palmitic acid as the main constituent (about 60 per cent.). D. G. H.

Chemical Examination of the Wax from Sugar Cane. N. L. Vidyarthi and M. Narasingarao. (*J. Indian Chem. Soc.*, 1939, **16**, 135–143.)—The wax isolated from the press cake from a sulphitation factory treating sugar cane had the following characteristics: m.p. 68·7° C.; saponification value, 133·5; iodine value, 31·5; acid value, 23·4; acetyl value, 89·6; unsaponifiable matter, 43·7 per cent. It consisted of 53·0 per cent. of acids which were examined by the ester fractionation method of Hilditch, and found to comprise resin acid, 4·5; caproic, 0·6; palmitic, 27·7; stearic, 22·4; oleic, 41·5; arachidic, 3·3 per cent. The unsaponifiable

part of the wax (43·7 per cent.) was treated by the phthalic anhydride method of Chibnall *et al.* (*Biochem. J.*, 1931, **25**, 2095; Abst., ANALYST, 1932, **57**, 258) and was resolved into primary and secondary alcohols and paraffins. The main portion (about 80 per cent.) was the primary myricyl alcohol, and about 10 per cent. consisted of a mixture of sterols, which by conversion into acetates and fractional crystallisation of the brominated products (Windaus and Hauth, *Ber.*, 1906, **39**, 4378) was found to contain brassicasterol, stigmasterol and sitosterol. About 5 per cent. of an aliphatic paraffin, *n*-pentatriacontane ($C_{35}H_{72}$), was separated but no dibasic or oxyacids could be detected. D. G. H.

Manganese-content of Indian Foodstuffs and Other Materials. M. N. Rudra. (*J. Indian Chem. Soc.*, 1939, **16**, 131–134.)—The manganese content of a number of Indian foodstuffs and some other materials has been determined by the Skinner and Petersen method (*J. Biol. Chem.*, 1930, **88**, 347; Abst., ANALYST, 1930, **55**, 640) with modifications where found necessary, and also in two instances by the Richards' method (ANALYST, 1930, **55**, 554). The manganese present in typical plant materials (mg. per 100 g. of dry substance) was: rice, sundried and polished, 1·34; parboiled, 1·96; wheat, 2·07; green gram, 1·93; Bengal gram, 3·00; cabbage, 5·69; amaranth, tender, 6·37; mature, 5·86; carrot, 0·754; knol-khol, 5·21; cauliflower, 2·62; brinjal, 2·40; guava, 1·59; chillies, green, 1·64; jujuba plum, 0·982; Indian gooseberry, 2·90. For animal products the figures were:—goat: liver, 1·233; muscle, 0·07; rohit fish: muscle, 0·248; scales, 8·831; calbosh fish muscle, 0·243; mrigal, catla and chital fish muscle, 0·2; milk, 0·0075 per 100 ml.; cows' milk, 0·006 per 100 ml. The plant products rich in manganese are also usually rich in vitamin C and *vice versa*, and the fact that the manganese-content of living materials runs almost parallel with their vitamin C content is illustrated by the figures for (*a*) tender and (*b*) mature amaranth leaves: manganese (mg. per 100 g. dry substance), (*a*) 6·37, (*b*) 5·86, vitamin C content (mg. per 100 g. of moist substance):—(*a*) 151, (*b*) 140. D. G. H.

Photometric Determination of Nicotine on Apples without Distillation. L. N. Markwood. (*J. Assoc. Off. Agr. Chem.*, 1939, **22**, 427–436.)—By the method previously described (*J. Assoc. Off. Agr. Chem.*, 1938, **21**, 151; Abst., ANALYST, 1938, **63**, 288), the nicotine on apples sprayed with nicotine bentonite is determined by distillation and conversion into the silicotungstate. The colour test for the determination of pyridine described by Barta (*Biochem. Z.*, 1935, **277**, 412) and shown later by Barta and Marschek (*Mëzogazdasagi Kutatasok*, 1937, **10**, 29) to be applicable, but with less sensitivity, as a test for nicotine can be used for the direct determination of nicotine on sprayed apples. The reagents are cyanogen bromide solution (freshly prepared by the addition of 10 per cent. potassium cyanide solution to saturated bromine water until it is decolorised and dilution of the product with water to five times its original volume), and a freshly prepared solution of 0·2 g. of β-naphthylamine in 100 ml. of 95 per cent. alcohol. This solution should not be exposed to sunlight, but a moderate fluorescence in daylight is normal. To prepare the standard comparison curve, 0·4 g. of nicotine bentonite is shaken with 400 ml. of 0·5 per cent. sodium hydroxide solution, 400 ml. of water and 20 ml. of calcium acetate solution containing 4 g. of calcium

per litre. The calcium compound serves to convert the bentonite into "calcium bentonite" which, unlike bentonite, is readily separated by filtration. The mixture is diluted to 1 litre, allowed to stand for 15 minutes and filtered. Aliquot portions of the filtrate (usually 50, 100, 150 and 200 ml.) are adjusted to the same alkali content as the largest by the addition of appropriate amounts of sodium hydroxide solution and, after the addition of phenolphthalein, the solutions are nearly neutralised with 30 per cent. acetic acid. Dilute acetic acid (2 per cent.) is then added until the indicator is colourless, one drop being added in excess. By this procedure the concentration of sodium acetate and the *p*H of the solution are fixed at the optimum values for the development of the colour. The solutions are diluted to 250 ml., and a 5-ml. portion of each solution is treated with exactly 1 ml. of cyanogen bromide solution, and, after mixing, with exactly 5 ml. of β-naphthylamine solution. The mixtures are placed in the dark for an hour, after which photometric measurements of the red or pink colour are made and a standard curve is constructed showing the relationship between the photometric reading and the concentration of nicotine. In accordance with Beer's law, a straight line was obtained for the range investigated, *viz.* 0 to 16γ. To determine the nicotine on sprayed apples, the fruit (about 10 mature apples) is shaken in a closed vessel first with 200 ml. of sodium hydroxide solution, then with 180 ml. of water, and finally with 80 ml. of water. The extracts are mixed, and 0·2 g. of powdered bentonite and 10 ml. of calcium acetate solution are added. The mixture, diluted to 500 ml., is filtered, and an aliquot portion (200 ml.) is treated as described for the standard solutions. The amount of nicotine present is determined from the curve. Lead arsenate, calcium arsenate, Bordeaux mixture, copper ammonium silicate, and "wettable sulphur" do not interfere with the process, but if cryolite is present, it is necessary to remove the fluoride ion from the alkaline solution by filtration after treatment with calcium oxide. Lime-sulphur caused the most interference of all added substances, the recovery of nicotine being only 86 per cent. when all forms of sulphur had been removed from the solution. It appears necessary in this instance to separate the nicotine by distillation before applying the method. Among organic compounds that may be present pyridine answers to the test, but its presence is unlikely, owing to its volatility. Its homologues respond only very feebly to the test. Nicotinic acid, which may be formed by oxidation of the nicotine, is removed as an insoluble calcium salt. Hitherto the method has been applied only to recently picked apples, and it is not claimed that the extraction will be equally complete with apples which have formed a thick coating of wax during storage. The accuracy of the method was tested by adding known amounts of nicotine bentonite to unsprayed apples. With a neutral wedge photometer equipped with a 10-cm. tube and a monochromatic filter transmitting around 490mμ, it was found possible to determine a nicotine concentration of 1 part in 4 millions in the final solutions. Determinations were made of the nicotine on single apples (about 0·8 mg.). A. O. J.

Identification of Strophanthus Species and their Tinctures. P. Dumont and G. Thomas. (*J. Pharm. Belg.*, 1939, **21**, 397–402.)—The commercial species of *Strophanthus* are three: (*a*) *S. kombe* from the East Coast of Africa, (*b*) *S. hispidus*

from the West African French Possessions, and (*c*) *S. gratus* or *glaber* from the Cameroons and Gaboon. In Belgium (*a*) and (*b*) are official, but (*c*) is used in other countries. The seeds of *S. gratus* may be distinguished from those of *S. kombe* and *S. hispidus* by being free from hairs, but magnification is necessary to distinguish between the long silky hairs of *S. kombe* and the short and close-set hairs of *S. hispidus*. The reactions given in the literature usually refer to *S. kombe* and *S. gratus*, whilst *S. hispidus* is ignored (*cf.*, however, Smelt, *Pharm. J.*, **1933**, **131**, 150–151; Abst., ANALYST, 1933, **58**, 704). Of the various distinguishing reactions investigated, that with furfural and sulphuric acid, and with vanillin in hydrochloric acid were found to be the most satisfactory and were studied in detail. In the former both *S. hispidus* and *S. kombe* tinctures give an indigo-blue colour which develops rapidly, and in proportion to the quantity of acid used, whilst *S. gratus* under similar conditions gives only a feeble rose colour, slowly intensifying towards violet. To distinguish *S. kombe* from *S. hispidus* the fluorescence, in ultra-violet light, of the ethereal solutions from the de-fatting of the powder preparatory to making the tincture may be examined. *S. kombe* shows an orange-red, *S. gratus* a quinine blue, and *S. hispidus* a pale lilac fluorescence. For the examination of tinctures, the alcohol is first completely evaporated on a water-bath, and the aqueous residue, after cooling, is shaken with 3 ml. of ether. The ethereal solution is shaken with 10 ml. of 0·1 *N* sodium hydroxide solution, and after separation of the two layers (by centrifuging if necessary), the aqueous layer is examined in ultra-violet light; *S. kombe* gives a blue, and *S. hispidus* and *S. gratus* a yellow-green fluorescence. D. G. H.

Biochemical

Nutritive Value of Wheaten Products. N. Palmer. (*Biochem. J.*, 1939, **33**, 853–858.)—The nutritive value of a mixed wheaten product consisting of 3 parts of wheat flour and 1 part of added germ was tested on a colony of albino rats. The abnormal calcium/phosphorus ratio (1 : 9) of the mixture was adjusted by addition of 3 per cent. of calcium lactate to produce a calcium/phosphorus ratio of 1:0·45. This modification effected a marked improvement in the nutritive value of the mixture, and a first generation of rats was reared and brought to sexual maturity on it. Some of the animals produced litters and in some instances suckled their young. This second generation was in turn reared on the diet and enabled to breed, but a third generation could not be reared on it. Adjustment of the calcium/phosphorus ratio of ordinary white flour did not remedy its lack of nutritive value. The flour-germ mixture with added calcium was superior to a meat diet with added calcium. A diet of bread and milk or bread and cheese gave excellent results, and rats fed on it grew rapidly and produced several litters. F. A. R.

Enzyme Activity in Frozen Vegetables. Stringbeans. C. M. Bedford and M. A. Joslin. (*Ind. Eng. Chem.*, 1939, **31**, 751–758.)—The investigation recorded was undertaken with the object of ascertaining the minimum temperature necessary in "blanching" stringbeans to destroy the enzymes, which otherwise

give rise to unpleasant flavours on storage even at −17° C. The activity of catalase, peroxidase and ascorbic acid oxidase and the acetaldehyde-content were studied. It was found that acetaldehyde, a known product of anaerobic respiration, accumulates in samples containing active catalase. The amount of acetaldehyde decreases with catalase activity, but there is no definite relation between the two, such as was found for peas, and the amount of the former and the activity of the latter are not a reliable index of flavour retention. The rate of heat inactivation of catalase was found to be more rapid than that of the agencies producing the flavours. The ascorbic acid oxidase activity runs closely parallel with that of catalase and is inactivated by heat at about the same rate. The peroxidase activity, as measured by the rate of decomposition of hydrogen peroxide in the presence of pyrogallol, is more resistant to heat, and there is close correlation between its inactivation and the keeping quality. It is difficult, however, to determine definitely from the data obtained how much of the peroxidase must be destroyed in order to secure good keeping quality; apparently it need not all be destroyed. The temperatures for "blanching" employed varied from 55° to 100° C., and the time from 30 seconds to 1 hour; the effect of these exposures upon the enzymes is recorded. A satisfactory product was obtained by "blanching" cut stringbeans for 2 minutes at 85° to 87·5° C., or for 5 minutes at 82·2° C., and for 2 minutes at 100° C. with less mature uncut stringbeans. D. R. W.

Effect of Calcium Ion on Tissue Respiration, with a Note on the Estimation of Oxaloacetic Acid. G. D. Greville. (*Biochem. J.*, 1939, **33**, 718–722.)—The inhibitory effect of calcium ions on the respiration of minced muscle is not localised in any particular enzymic reaction, since both the formation and removal of oxaloacetic acid, and also the accumulation of succinic acid, were shown to be inhibited by the presence of calcium ions. The experiments by which this was demonstrated required a method of estimating oxaloacetic acid. In Ostern's method (*Z. physiol. Chem.*, 1933, **218**, 160) the carbon dioxide evolved when aniline reacts with oxaloacetic acid is measured in a Warburg apparatus, but the reaction is slow, since it has to be carried out at 5° C. to avoid decomposition of the acid prior to reaction with aniline. Two methods were used to increase the rate of reaction. In the first method aniline citrate was employed—a reagent previously used by Edson (*Biochem. J.*, 1935, **29**, 2082) in the estimation of acetoacetic acid at 25° C. The aniline citrate solution is added to the oxaloacetic acid solution from the side-bulb at 5° C., and the reaction is complete in 10 to 20 minutes. A correction has to be applied for the solubility of carbon dioxide in the mixture in the vessel. Duplicate estimations differed by not more than 2·5 per cent., and 97 per cent. purity was indicated by the method for three different oxaloacetic acid preparations. In the second method the bulb contains 0·2 ml. of aniline previously mixed with 0·15 ml. of conc. hydrochloric acid, whilst the main part of the apparatus contains a mixture of 1·5 ml. of absolute alcohol, 0·3 ml. of acetate buffer solution (0·3 *N* sodium acetate solution and 2·7 *N* acetic acid) and 0·86 ml. of the solution to be analysed. Again, a correction must be applied for the solubility of carbon dioxide. Results were obtained agreeing closely with those given by the first method. Whereas the alcohol method does

not distinguish between oxaloacetic acid and acetoacetic acid, the citrate method at 5° C. does, and oxaloacetic acid can be estimated in the presence of acetoacetic acid. The evolution of carbon dioxide from the latter is slow and fairly constant, and extrapolation to zero time gives the amount of oxaloacetic acid with reasonable accuracy. F. A. R.

Fermentation Process in Tea Manufacture. I. Rôle of Peroxidase. E. A. H. Roberts and S. N. Sarma. (*Biochem. J.*, 1938, **32**, 1819–1828.)—The processing of tea is briefly as follows:—After plucking, the tea-leaf is first spread out in thin layers to undergo withering, and is then subjected to a rolling process, the primary purpose of which is to rupture the leaf-cells and expose the expressed juices to the air. At the same time the tea receives its characteristic twist. It is at this stage that fermentation begins, and after 1½ hours' rolling fermentation is continued on the fermenting floor for a further 2 hours, after which the tea is fired to stop fermentation and dry the leaf. It is now certain that the process of fermentation is an oxidation by atmospheric oxygen catalysed by peroxidases in the leaf. The first stage is an uptake of oxygen leading to the oxidation of some as yet unidentified substance and the production of hydrogen peroxide. The peroxidase then catalyses the oxidation of the tea tannin by means of this hydrogen peroxide, leading to the production of brown pigments which impart the characteristic colour to a tea infusion. The "non-tans" (*i.e.* matter oxidised by potassium permanganate, but not precipitated by gelatin and acid salt) also appear to undergo oxidation during fermentation. No evidence could be obtained that organic peroxides are utilised by the peroxidase in place of hydrogen peroxide. Tea tannin inhibits peroxidase action, but substances present in the leaf exert some protective influence against its effects. The authors devised a method of estimating peroxidase activity in tea-leaf, based on that of Guthrie (*J. Amer. Chem. Soc.*, 1931, **53**, 242; Abst., ANALYST, 1931, **56**, 194). A solution of 1·05 g. of crystalline citric acid in 8·5 ml. of *N* sodium hydroxide solution was diluted to 100 ml. with water, and 0·25 g. of *p*-phenylenediamine hydrochloride and 5 ml. of a 4 per cent. solution of α-naphthol in 50 per cent. alcohol were added. The solution was then filtered. Ten ml. of this reagent were mixed with the enzyme solution (2 ml.) and 2 ml. of 0·05 *N* hydrogen peroxide solution were added. After 10 minutes' incubation the reaction was stopped by the addition of 2 ml. of 1 per cent. potassium cyanide solution. An aliquot portion (usually 3 ml.) was extracted with 25 ml. of toluene, and the colour of the toluene layer was measured in a Lovibond tintometer (red and blue glasses). A solution was said to have 6 Indophenol units (I.U.) when the total reading of the red and blue slides was 6·0. The peroxide-content of an unknown solution was calculated from a calibration curve. Since peroxidase activity is inhibited by tannins, the latter have to be removed before the estimation can be carried out. The leaf sample (10 g.) was therefore ground with sand under alcohol, the alcoholic suspension was filtered, and the residue was washed, dried and extracted with 100 ml. of *M*/15 phosphate buffer solution (*p*H 7·0). The method was employed to follow the variations in peroxidase-content that occur during the process of manufacture. An increase was found after withering, followed by a marked decrease after the first rolling, with a slight though regular

decrease in the second rolling and the fermentation. The firing did not completely destroy the peroxidase, for 0·5 to 1 per cent. of the original activity remained. This may be of some importance in the post-fermentative changes that take place on maturing. F. A. R.

Fermentation Process in Tea Manufacture. II. Some Properties of Tea Peroxidase. III. Mechanism of Fermentation. E. A. H. Roberts. (*Biochem. J.*, 1939, **33**, 836–852.)—II.. The peroxidase-content of fresh tea leaves, estimated by the method previously described (*cf.* preceding abstract), was found to vary widely in single shoots (and even in different parts of a shoot) from the same bush and in samples from different bushes on the same plot. Representative samples showed regular variations in the peroxidase activity from week to week. In the curve in which these variations were plotted, three well-defined regions were observed, corresponding closely with three quite sharply contrasted types of tea. Little correlation, however, was found between peroxidase activity and the rate of fermentation (peroxidase-catalysed oxidation of tannin); this suggests that peroxidase is not the sole factor governing the rate. The peroxidase activity was found to increase during the withering process and was accelerated by loss of moisture until a critical level (72 per cent.) was reached, after which there was a decrease. Estimations of the rate of fermentation in fresh and withered leaf were carried out by measuring both the rate of oxygen uptake (see Part III) and the rate of decrease of the tannin titre, and by neither method was any significant difference observed between the fermentation rates for green and for withered leaf. Protein nitrogen was found to decrease during withering. As the rate of oxidation of tannins appears to be quite independent of the peroxidase activity, it is concluded that the oxidation is preceded by a relatively much slower reaction in which hydrogen peroxide is formed, and that the rate of fermentation depends on the rate of hydrogen peroxide formation. It is further suggested that the hydrogen peroxide required is derived from oxidation of the "non-tans" during fermentation. The proposed mechanism of fermentation can thus be represented by the equations:

(1) "Non-tan" + O_2 $\longrightarrow$ oxidised "non-tan" + H_2O_2.

(2) H_2O_2 + tea tannin $\xrightarrow{\text{peroxidase}}$ oxidised tea-tannin.

III. Estimations of the rates of oxygen uptake by fermenting tea-leaf were made by the Barcroft-Warburg method, 200 mg. of the finely-minced leaf being suspended in 3 ml. of water and the reaction vessel shaken vigorously in a thermostat maintained at 35·5° C. The uptakes were the same over a *p*H range of 4·6 to 6·6 and were initially quite rapid, but fell off when the substrate was exhausted. At this stage about 80 per cent. of the original enzyme activity was still present. Carbon dioxide was also produced during fermentation, presumably by oxidation of carbohydrate. After allowance had been made for the oxygen required in the latter reaction, it was calculated that one atom of oxygen is required for the complete oxidation of one molecule of tea tannin. Since carbon dioxide was produced in amounts equivalent to about half the total oxygen uptake, it seemed probable that the "non-tans" oxidised during fermentation were carbohydrates,

and this was confirmed by the identification of glucose as the major constituent in a tannin-free extract of the "non-tans." Estimations of the ascorbic acid content of green leaf were made, after removal of tannin with neutral lead acetate, by titration with 0·01 *N* iodine solution after the addition of 4 ml. of 12 *N* sulphuric acid. It was found that 1 g. of fresh green leaf contains about 1 mg. of ascorbic acid. A thermolabile catalyst of ascorbic acid oxidation appears to be present in the leaf. The addition of ascorbic acid to fermenting leaf had no effect on the rate of oxygen uptake, but the rate was maintained for a longer period, the ascorbic acid being oxidised before either the tannin or the carbohydrate is attacked. Since, however, the rate of oxygen uptake during the oxidation of ascorbic acid is identical with the rate of normal fermentation, it is evident that the latter is controlled by the former. This, however, is the same as the rate of production of hydrogen peroxide, which is required for the peroxidase-catalysed oxidation of tannin. A shortage of ascorbic acid oxidase, as occurs in Kharikatia leaf, results in a slower rate of fermentation. The dehydroascorbic acid formed by oxidation of ascorbic acid probably serves as a hydrogen acceptor during the oxidative breakdown of carbohydrate, ascorbic acid being regenerated only to be re-oxidised and the cycle repeated. About 20 moles of tea tannin are oxidised for each mole of ascorbic acid present. The primary product of tannin oxidation is probably an *o*-quinone which either condenses irreversibly or is again reduced to tea tannin by ascorbic acid. The complete reaction scheme for fermentation can be summarised as follows:

(1) Ascorbic acid + O_2 $\xrightarrow{\text{ascorbic acid oxidase}}$ dehydroascorbic acid + H_2O_2.

(2) H_2O_2 + tea tannin $\xrightarrow{\text{peroxidase}}$ tannin *o*-quinone.

(3) *o*-Quinone ⟶ condensation products.

(4) *o*-Quinone + ascorbic acid ⟶ tea tannin + dehydro-ascorbic acid.

(5) Dehydroascorbic acid + glucose $\xrightarrow{\text{zymase}}$ CO_2 + ascorbic acid.

F. A. R.

Vitamin G Content of some Oil Presscake Meals and Related Products. F. W. Sherwood. (*J. Agric. Res.*, 1939, **58**, 787–794.)—The term "vitamin G" is used to denote the undifferentiated complex which stimulates growth of rats on a basal ration containing maize starch and extract of rice polishings, but otherwise free from water-soluble vitamins, riboflavin being probably the chief limiting factor of the diet. Peanut, cottonseed, linseed and soya bean meals, as well as cottonseed flour and cottonseed hull bran, and soya beans, were fed to rats previously maintained on the basal ration (devoid of the vitamin B complex) with 2 drops of extract of rice polishings daily until they ceased to gain in weight, and standard yeast was given for comparison to a further batch of rats that had been similarly fed. The optimum test period was found to be about 7 weeks. All the products were found to be good sources of vitamin G, the values ranging from 2·5 to 5·9 Sherman–Bourquin units per gram, which is equivalent to the vitamin G content of cheddar cheese and green leafy vegetables. Time of year and, to a less

extent, initial weight of the rat and gain in the depletion period, affected the relation between the gain in weight and vitamin G intake, and a curve of reference with corrections is given. D. G. H.

Biological Estimation of Crystalline Vitamin B_1. K. H. Coward and B. G. E. Morgan. (*Biochem. J.*, 1939, **33**, 658–662.)—Kinnersley and Peters (*Biochem. J.*, 1936, **30**, 985) measured the effect of graded doses of vitamin B_1 by the duration of the cure of "retracted neck" in pigeons, and came to the conclusion that with crystalline vitamin B_1 there was no relation between the effect and the dose. When the effect was measured by the percentage of birds cured, however, the relationship between effect and dose was more satisfactory. The response of pigeons to graded doses of crystalline vitamin B_1 given orally has been re-investigated with the following results:

Expt.	Dose mg.	No. of birds	Birds cured Per Cent.	Average duration of cure Days
a	5·0	15	33·3	3·6
	10·0	15	66·7	4·3
b	4·5	22	54·5	6·6
	9·0	22	81·8	5·5
	13·5	22	90·9	6·2
c	3·0	22	59·1	6·2
	6·0	22	50·0	8·7
	9·0	22	77·3	7·4
d	1·0	25	8·0	5·5
	3·0	25	28·0	5·6
	9·0	25	56·0	5·4

From the above table it is clear that there is a direct relationship between the dose and the percentage of birds cured, but not between the dose and the duration of cure; this confirms the earlier work of Kinnersley and Peters. The accuracy of the method is greater, the larger the number or birds used, the more nearly the number of birds cured approaches 50 per cent., and the steeper the response curve. Statistical examination of the results shows that the pigeon method of assay is the least accurate of those investigated by the Sub-Committee on the Accuracy of Biological Assays, British Pharmacopoeia Commission. F. A. R.

Bacteriological

Carbon Monoxide in Underground Atmospheres. The Role of Bacteria in its Elimination. G. W. Jones and G. S. Scott. (*J. Ind. Eng. Chem.*, 1939, **31**, 774–778.)—Tests are described showing that certain types of bacteria occurring in gangway waters, sewage and surface materials are capable of consuming hydrogen and carbon monoxide from gaseous mixtures. The reactions involved appear to be largely oxidation of carbon monoxide to dioxide and of hydrogen to water. In the tests in which oxygen was present in the atmosphere the carbon monoxide did not combine with the hydrogen to form methane and water vapour. Under favourable conditions certain anthracite mine micro-organisms remove carbon monoxide rapidly; one gangway water, for instance,

contained sufficient bacteria to remove 1·7 volumes of carbon monoxide per volume of water in 20 days. The types of bacteria to which these reactions are due has not yet been ascertained, but investigations in this direction are in progress.* Experimentally prepared atmospheres containing carbon monoxide and hydrogen were exposed, in a closed glass vessel, to a sponge holding gangway water, and analyses of the mixed gases were made at the beginning of the experiment and after 30 or 40 days. The results obtained in one typical experiment were as follows:

	CO_2	O_2	H_2	CO	CH_4	N_2
Percentage at beginning ..	0·4	11·3	14·3	12·3	0	61·7
,, after 30 days ..	7·0	4·7	1·6	7·6	0	79·1

It is suggested that it is unsafe to assume that anthracite mine fires are extinguished when the atmosphere in a sealed fire area contains no carbon monoxide, for this gas may be eliminated as fast as it is generated. D. R. W.

Bactericidal Properties of Commercial Antiseptics. Effect of *p*H. W. A. Bittenbender, E. F. Degering and P. A. Tetrault. (*Ind. Eng. Chem.*, 1939, **31**, 742–744.)—The bactericidal properties of phenylmercuric nitrate, merthiolate, metaphen, mercurochrome, tincture of iodine, phenol, hexylresorcinol and acriflavine were tested under controlled conditions of *p*H against *Staphylococcus aureus* and *B. coli*. Metaphen and mercurochrome could not be adjusted to the desired *p*H values, and acriflavine gave negative results. All the others showed increased potency as the hydrogen-ion concentration of the medium increased. Phenylmercuric nitrate, for example, was effective in a dilution 1 in 10,000 at *p*H 7·0, and at *p*H 3·0 its potency against *Staphylococcus aureus* was increased tenfold. At *p*H 4·0 phenol showed a 25 per cent. increase as compared with its effect at 7·0. The results of the investigation indicate that, except for interfering factors such as increased insolubility or instability, these antiseptics of widely differing types show a definite increase in effectiveness with an increase in the hydrogen-ion concentration. This is in agreement with the findings of Goshorn, Degering and Tetrault in their studies of the phenylalkanoic acids. Experimental results are set out in tabular form. The culture was adjusted to the *p*H of each test before use. The concentrations indicated in the table killed in 10, but not in 5, minutes at 37° C. In the following abridged table the lowest killing concentrations at extreme *p*H values are shown.

	Staphylococcus aureus		*B. coli*	
	*p*H 3	*p*H 7	*p*H 3	*p*H 7
Phenylmercuric nitrate..	1 in 100,000	1 in 10,000	1 in 400,000	1 in 30,000
Merthiolate	1 ,, 75,000	1 ,, 10,000	1 ,, 100,000	1 ,, 5,000
Tincture of iodine ..	1 ,, 4,000	1 ,, 1,000	1 ,, 4,000	1 ,, 1,500
Phenol	1 ,, 90	1 ,, 60	1 ,, 100	1 ,, 75
Hexylresorcinol ..	1 ,, 100,000	1 ,, 3,000	1 ,, 100,000	1 ,, 5,000

Killing dilutions for intermediate values and those for metaphen and mercurochrome at *p*H 10·0 are also shown. D. R. W.

* It will be of interest to learn whether *B. pantotrophus*, *B. pycnoticus* and *B. oligocarbophilus* are found.—D. R. W.

Water

Reaction of Carbonic Acid with the Zeolite in a Water Softener. Roy E. King and O. M. Smith. (*Ind. Eng. Chem.*, 1939, **31**, 727–8.)—It is shown that as softening by zeolite proceeds there is a gradual decrease of alkalinity, with the formation of hydrogen zeolite, which subsequently exchanges its hydrogen ion with basic ions and thus forms free carbon dioxide and lowers the *p*H of the effluent water. It was noted that no matter how narrow the range of the alkalinity of a water before treatment there was a wide variation in the alkalinity of the effluent from the zeolite filter, and the investigation recorded was consequently undertaken. Two waters of practically the same composition were in vestigated (full analyses are given); they showed (in parts per 100,000) 20·6 total hardness as $CaCO_3$, 19·0 alkalinity, 1·8 chloride and 3·3 sulphate, but one contained 1·5 part of free carbon dioxide and the other only 0·7. Graphs are given showing the changes in the alkalinity, carbon dioxide content and *p*H of the effluent waters from a zeolite filter at half-hourly intervals for 20 hours. These show at the end of the run:

No. 1 (*original free* CO_2 1·5) alkalinity 17·0, free CO_2 3·5, *p*H 6·6; *No.* 2 (*original* CO_2 0·7) alkalinity 16·0, free CO_2 2·2 and no normal carbonate, whereas at the beginning of the run there was no free carbon dioxide in either water and the *p*H of No. 1 was 7·9. The authors believe that the reactions that occur are as follows:

$$H_2CO_3 + 2\text{Na Zeolite} \longrightarrow Na_2CO_3 + 2\text{H Zeolite}$$

and $$H_2CO_3 + \text{Na Zeolite} \longrightarrow NaHCO_3 + \text{H Zeolite},$$

and that as softening progresses the hydrogen zeolite reacts with the calcium and magnesium bicarbonates as follows: $Ca(HCO_3)_2 + 2\text{H Zeolite} \longrightarrow CaZ_2 + 2H_2CO_3$. The following experiments were made to check these assumptions. Carbon dioxide gas was passed through distilled water until the *p*H was 6·8. The water was then allowed to percolate through a small quantity of greensand zeolite. The effluent had *p*H 9·0 and contained only carbonate alkalinity to the extent of 21 p.p.m. as sodium carbonate. In a further experiment carbon dioxide was passed through distilled water until the *p*H was lowered to 4·9. When this water was allowed to percolate through the zeolite bed, the *p*H increased to 7·9 and only bicarbonate alkalinity appeared to the amount of 11·0 p.p.10^5 as sodium carbonate. Then when sufficient carbonated distilled water had been passed through the zeolite bed so that no further change occurred, tap water with *p*H 7.5 and a total alkalinity of 19·0 p.p.10^5 was substituted for the carbonated water. The effluent water had *p*H 4·4 after it had been heated and aerated to drive off any carbon dioxide; the formation of hydrogen ions was thus indicated. D. R. W.

Agricultural

Determination of Thallous Sulphate in Ant Poisons. C. G. Donovan. (*J. Assoc. Off. Agr. Chem.*, 1939, **22**, 411–414.)—Of the methods available for the determination of thallium, the most applicable to its determination in ant poisons appears to be that based upon its conversion into thallous iodide provided that the

conditions are such that the solubility of the precipitated thallous iodide is reduced to a minimum. This compound is slightly soluble in pure water (0·0847 g. per litre at 26° C.), but is much less soluble in solutions of potassium iodide, alcohol or acetic acid and is almost insoluble in water containing ammonia or sodium thiosulphate. A quantity of the sample containing 0·1 to 0·15 g. of thallous sulphate (usually about 10 g.) is heated in a Kjeldahl flask with 25 ml. of conc. sulphuric acid and 5 ml. of conc. nitric acid until the organic matter has been destroyed. The cold liquid is diluted with 10 to 15 ml. of water, washed into a beaker with water until the volume is 60 to 70 ml., boiled for several minutes and filtered, and the filter is washed with hot water until the filtrate measures 175 ml. The filtrate is neutralised with ammonia and, after slight acidification with dilute sulphuric acid, is treated with 1 g. of sodium bisulphite to ensure complete absence of thallic salts. After the addition of 50 ml. of 10 per cent. potassium iodide solution, the mixture is allowed to stand overnight. The precipitated thallous iodide is collected on a Gooch crucible containing two paper discs (S. & S., No. 589) covered with asbestos, and is washed four or five times with potassium iodide solution and finally with absolute alcohol. The crucible is dried to constant weight at 105° C., and the amount of thallous iodide found is calculated to thallous sulphate (factor: 0·7616). The accuracy of the method was proved by the determination of known amounts of thallous sulphate added to such substances as honey, sugar, walnut meal and peat moss in imitation of the commercial products. A. O. J.

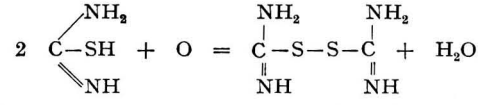
Organic

***p*-Xenylsemicarbazide as a Reagent for the Identification of Aldehydes and Ketones. P. P. T. Sah and I. S. Kao.** (*Rec. Trav. Chim. Pays-Bas*, 1939, **58**, 459–464.)—*p*-Xenylsemicarbazide gives with carbonyl compounds *p*-xenylsemicarbazones which can be used for the identification of the carbonyl compounds; the reagent allows of the rapid differentiation of normal aliphatic aldehydes from C_2 to C_{10}, which cannot easily be identified by semicarbazones formed from semicarbazide. *p*-Xenylsemicarbazide is prepared by heating under reflux for 24 hours a mixture of *p*-xenylurea, hydrazine hydrate and absolute ethyl alcohol, removing the solvent by distillation, evaporating to dryness, and recrystallising the residue from 95 per cent. ethyl alcohol after washing with ice-cold water. For the identification of aldehydes and ketones, 0·5 g. of *p*-xenylsemicarbazide is dissolved in 5 ml. of 95 per cent. ethyl alcohol, and the solution is acidified with a few drops of glacial acetic acid. A molecular equivalent of the carbonyl compound is dissolved in 5 ml. of 95 per cent. ethyl alcohol, and the solution is added to the semicarbazide solution. The mixture is heated under reflux on the water-bath for a few minutes and filtered hot, and the filtrate is allowed to stand. The semicarbazone generally crystallises out, but if its solubility is too high the solution is concentrated to half the original volume and then allowed to crystallise. The crystals are filtered off with the aid of suction, the m.p. is determined and the crystalline form examined, and the semicarbazone is then recrystallised (usually from 95 per cent. ethyl alcohol, sometimes from ethyl acetate or petroleum spirit) until the m.p. is constant. Tables show the m.p., crystalline forms and nitrogen-contents of a number of

p-xenylsemicarbazones, and the m.p. of the phenyl-, *p*-tolyl-, *o*-tolyl-, *m*-tolyl-α-naphthyl-, β-naphthyl-, 3·5-dinitrophenyl-, *p*-xenyl-, and 2.4-dinitrophenyl-semicarbazones from a number of carbonyl compounds. E. M. P.

β-Naphthazide and β-Naphthyl Isocyanate as Reagents for the Identification of Phenols. P. P. T. Sah. (*Rec. Trav. Chim. Pays-Bas*, 1939, **58**, 453–458.)—Both β-naphthazide and β-naphthyl isocyanate react with phenolic compounds in boiling petroleum spirit to give, almost quantitatively, crystalline urethanes, the properties of which can be used to characterise the phenols. One-hundredth of a mole of β-naphthyl isocyanate (0·17 g.) or an equivalent quantity of β-naphthazide is weighed into an Erlenmeyer flask containing 5 ml. of dry petroleum spirit and an equivalent molecular quantity of the phenol to be tested. The mixture is gently heated under reflux on the sand-bath for 4 hours and then allowed to stand overnight in a cold place; the urethane crystallises out and can be filtered off by suction. The product is weighed, its m.p. determined, and its crystalline form examined. The yield is generally nearly the theoretical. The m.p. becomes constant after 1 or 2 recrystallisations from petroleum spirit. The nitrogen-content of the recrystallised urethane is determined by Kjeldahl's method. Tables give the physical properties and nitrogen-contents of a number of β-naphthyl urethanes in comparison with the m.p. of isomeric α- and β-naphthylurethanes from a number of phenols. E. M. P.

Volumetric Determination of Thiourea. C. Mahr. (*Z. anal. Chem.*, 1939, **117**, 91–94.)—In presence of sufficient potassium iodide, potassium bromate reacts with thiourea according to the equation



After this reaction is complete iodine is liberated. In absence of iodide, further oxidation to urea and sulphate occurs before the end of the disulphide reaction. With too high a concentration of iodide, iodine is liberated by the disulphide before the end-point of the titration is reached; hence this is not sharp. The following method for the determination of thiourea is simple and accurate:—The thiourea solution is mixed with 30 ml. of sulphuric acid (1: 1), 5 ml. of 1 per cent. potassium iodide solution and a little starch solution and diluted to about 80 ml.; then, while being shaken round, it is titrated at about 35° C. with *N*/10 potassium bromate solution containing potassium bromide, until a clear blue colour appears. If, after addition of 20 ml. of bromate solution, the reaction is not complete, 1 ml. more of the iodide solution is added. On the appearance of a permanent blue colour the mixture is diluted to 250 ml. with water at about 35° C., which decolorises it, and is then titrated, drop by drop, to the final blue end-point. Test analyses show that the titration can also be carried out in hydrochloric and perchloric acids. Small amounts of nitrate, corresponding with amounts up to about 10 ml. of 2*N* nitric acid, have no effect on the results, but higher concentrations are harmful, as are also copper and mercury salts. Interference by ferric salts is prevented by adding phosphoric acid. E. B. D.

Determination of Tetraethyl Lead in Gasoline. G. Calingaert and C. M. Gambrill. (*Ind. Eng. Chem.. Anal. Ed.*, 1939, **11**, 324–325.)—A 100-ml. sample is heated under a reflux condenser with 50 ml. of conc. hydrochloric acid (sp.gr. 1·19) for 30 minutes. After cooling, the acid layer is separated, the gasoline is washed with 50 ml. of water and the aqueous liquid is evaporated to dryness. Thirty ml. of nitric acid are added to the residual lead chloride, and the liquid is evaporated to dryness to oxidise organic matter. The lead salt is finally dissolved in dilute nitric acid, and the lead is determined by any convenient method: the molybdate titration method with tannin used as external indicator, or the lead chromate gravimetric method, was employed by the authors. With highly volatile gasoline (75 per cent. with b.p. below 100° C.) it is recommended that a 50-ml. sample be used and mixed with 50 ml. of a lead-free sample of higher boiling range (*e.g.* 10 per cent. at 204° C.; 90 per cent. at 238° C.), otherwise the recovery of lead tends to be a little low (by 0·05 to 0·1 ml. of tetraethyl lead per gallon). A convenient all-glass apparatus incorporating an electrical heating unit and separatory funnel is described. S. G. C.

Hydrogenation of Castor Oil with Nickel Catalysts containing Manganese, Zinc or Thorium. K. Kino. (*J. Soc. Chem. Ind., Japan*, 1939, **42**, 189B.)—Castor oil was hydrogenated at a temperature of 200° C. with the following catalysts: (*a*) nickel 10 parts; (*b*) nickel 5, manganese 5; (*c*) nickel 10, zinc 5; (*d*) nickel 10, thorium 5 parts. With nickel alone, iodine and hydroxyl values were much lowered (in 1 hour to 17·8 and 79·5 respectively). Nickel and thorium behaved like nickel alone; the addition of zinc to nickel weakened the hydrogenation of the double bond and reducing action of the ·OH group, whilst with nickel and thorium both actions were stronger than with nickel alone (in 1 hour the iodine value was reduced to 3·2 and the hydroxyl value to 46). D. G. H.

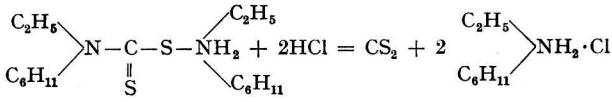
Temperatures of Crystalline Deposition of Stearone and Carnauba Wax from their Solutions in Various Organic Liquids. K. Kino. (*J. Soc. Chem. Ind. Japan*, 1939, **42**, 186–187B.)—Stearone, m.p. 87–88° C., prepared from pure stearic acid and recrystallised from butyl alcohol, and carnauba wax, m.p. 84–85° C., purified by solution in benzene and treatment with activated charcoal, were dissolved in a wide range of organic liquids in the proportion of 0·2, 0·4, 1·0, 2·0, 4·0, 6·0 and 10 per cent., and the temperatures of crystalline deposition were noted. Results were similar for both substances, although, on the whole, stearone was less soluble than carnauba wax. The lowest deposition temperatures were obtained with aromatic hydrocarbons, chloroform and carbon tetrachloride solutions, the next lowest with turpentine oil, hydro-aromatic hydrocarbons, cyclic ketones and acetic esters, and the highest with dibutyl phthalate, castor oil and ethyleneglycol monoethylether acetate. A full table of results is given. D. G. H.

***Cochlospermum tinctorium*, A. Rich. J. Rabaté.** (*J. Pharm. Chim.*, 1939, **29**, 582–583.)—The roots of this member of the *Bixaceae* family, which occurs in the dry regions of French East Africa, are used locally as a source of colouring matters. The samples examined by the author consisted of friable

fragments of large rhizomes (length, 10 cm., diameter 4 to 5 cm.) and finer disc-shaped pieces (diameter 2 to 4 cm., thickness 1 cm.); the surface of the latter was yellowish-white, owing to the presence of an orange exudate, but, on scratching, it acquired the white appearance of starch. The starch was present as irregular grains of unequal size (10 to 30μ), as a rule bell-shaped on one side and cut off sharply on the other, but having occasionally three faces. They were frequently grouped in pairs, and in appearance closely resembled manioc starch, the hilum being indistinct, although a central star-shaped fissure was apparent in the burst grains. The starch was determined by extracting the pulverised sample with ether in a Soxhlet apparatus until all the colouring matter had been removed. The residue was then dried in air and hydrolysed with a preparation of pancreatic diastase (*cf.* Bourdouil, *Bull. Soc. Chim. Biol.*, 1931, **13**, 809). The large roots contained 41·4 per cent., and the discs 56·5 per cent. of anhydrous starch. The starch was extracted by shaking 50 g. of the powdered sample with 2 litres of water, and then separating the fibres from the starch on a No. 120 sieve with the aid of water. The starch, which settled rapidly and could be washed by decantation, was finally collected on cloth and dried in air (yield, 50 per cent. on the dry weight of the original material); it was then pale grey in colour. Attempts to extract and identify the colouring matter were unsuccessful. J. G.

Inorganic

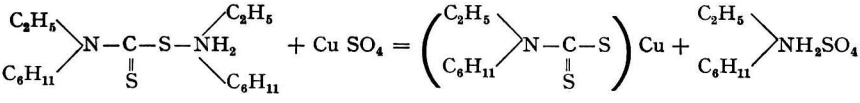
Analytical Applications of Dimine. M. Herrmann-Gurfinkel. (*Bull. Soc. Chim. Belg.*, 1939, **48**, 94–103.)—The dithiocarbamate of cyclohexylethylamine, "dimine," used as a catalyst in the vulcanisation of rubber, is a crystalline substance, m.p. 93°, which volatilises with decomposition above 100° C.; solubility in water, about 5 g. per litre at 20° C. and 10 g. per litre at 80° C. It is decomposed, with evolution of carbon disulphide, by dilute mineral acids:



With the salts of most metals, in neutral solution and in normal hydrochloric or sulphuric acid, dimine forms very bulky precipitates, the colours of which are as follows:—White: Pb, Hg^{II}, As^{III}, As^{V}, Sb^{III}, Sn^{II} (in neutral solution), Zn; Yellowish-white: Cd, Sb^{V}; Yellow: Ag, Bi (canary-yellow), Sn^{II} (in acid solution), Sn^{IV} (turning brown in air); Brown: Cu, Fe^{II} (greyish-brown), Fe^{III} (dark brown); Green: Ni (bright), Co (dark); Greyish-black: Hg^{I}. Manganous salts, which do not form precipitates in acid solution, give a brown one in neutral solution; molybdates in neutral solution give a yellow precipitate, which turns brownish-pink after addition of salts of zinc or tervalent bismuth and red after addition of hydrochloric or sulphuric acid or of aluminium salts; the uranyl ion, UO_2^{++}, forms a brick-red precipitate with dimine. Silver, lead, copper and zinc are also precipitated in *N* potassium hydroxide solution, and cadmium, nickel, cobalt and manganese in *N* ammonium hydroxide solution. The formation of a turbidity or colour with the reagent in very dilute solution is a very sensitive test for some metals. The

limiting concentrations, in gram-atoms per litre, at which these were perceptible were approximately:—Colours: Cu $4\cdot10^{-6}$ (golden yellow); Fe^{III} $2\cdot10^{-5}$, Bi^{III} $4\cdot10^{-5}$, Ag $3\cdot10^{-4}$ (yellow); Mn $4\cdot10^{-5}$ (pink); Ni $4\cdot10^{-5}$ (greenish); Co $4\cdot10^{-5}$ (green); white turbidities: Sb^{III} $3\cdot10^{-5}$, As^{III} $3\cdot10^{-4}$, Cd $4\cdot10^{-4}$, Zn $4\cdot10^{-4}$. The test was made by adding 3 drops of a 0·5 per cent. dimine solution to 3 ml. of a dilute solution of the metallic chloride. Dimine is a much more sensitive reagent than hydrogen sulphide for the detection of copper, and traces of silver in solution can be detected by adding a few drops of dimine solution followed by a few drops of conc. ammonium persulphate solution; in presence of silver a blue colour, rapidly changing through green to yellow, is obtained.

Solutions of dimine can be standardised by titration with copper sulphate solution and can then be used for the determination of copper. A 0·8 to 0·9 per cent. dimine solution which has been prepared at 80° C. remains clear, when cold, for two or three days, and its strength, as determined by titration, does not alter for some weeks, in spite of the turbidity which forms through separation of the reagent in this supersaturated solution. The brown precipitate formed with copper sulphate solution coagulates and settles almost instantaneously, leaving the supernatant liquid clear, and the end-point of the reaction, when precipitation ceases, is clearly marked. The reaction is:



Copper may be also determined gravimetrically with dimine, the precipitate being dried, ignited and weighed as copper oxide. Test analyses with known weights, excess of dimine being used, gave percentage errors of +0·5, +0·1 and −0·5, respectively, the corresponding excess of dimine being 50, 10 and 10 per cent.

Iron has also been determined gravimetrically in test solutions of ferric chloride in which the iron, determined by the nitrite method, was equivalent to 0·0937 g. of Fe_2O_3 in 10 ml. Ten ml. of the solution were treated with 10 ml. of 10 per cent. ammonium chloride solution and, after dilution to 100 ml., with 140 to 190 ml. of a 0·8 per cent. solution of dimine, added, drop by drop, until an excess of 10 to 50 per cent. was present. The precipitate was filtered off, washed with about 400 ml. of 0·08 per cent. dimine solution, dried at 70° C., and ignited carefully until combustion of organic matter was complete. The residue was taken up with a few drops of nitric acid, the solution was evaporated, and the ferric oxide is re-ignited and weighed. In three out of four determinations results were 0·6 per cent. above theory.

Dimine may be used for the separation of iron from chromium without conversion of chromic salts into chromates. Chromium is precipitated by dimine only in very concentrated neutral solutions; this precipitation is prevented by the presence of ammonium chloride. Determinations, made by the method described, on 100-ml. samples containing 10 ml. of $M/10$ ferric chloride, 10 ml. of $M/10$ chromic chloride and 1 g. of ammonium chloride and variable amounts of hydrochloric acid show that the optimum concentration of acid for the analysis is $N/20$, but most of these results were slightly too high and there appears to be a constant

source of error in the determination. For complete precipitation of the iron, excess of dimine sufficient to neutralise the acid present is required. The washing of the precipitate with 0·08 per cent. dimine is continued until all chlorides are removed; this requires approximately 500 ml. E. B. D.

Iodimetric Determination of Copper. S. K. Hagen. (*Z. anal. Chem.*, 1939, **117**, 26–30.)—The following process combines speed and economy while preventing the interference of nitrous compounds. A quantity of alloy containing about 0·2 g. of copper is dissolved in 10 ml. of nitric acid (1 : 4). The solution is boiled until the red fumes have been expelled, treated with 10 ml. of a solution containing 1·5 g. of lead nitrate, 100 g. of urea, and a little nitric acid per litre, stirred vigorously, and cooled to room temperature. After addition of 10 ml. of 10 per cent. potassium thiocyanate and 10 ml. of 1 per cent. potassium iodide solution, the solution is shaken and titrated at once with thiosulphate solution standardised against pure copper, starch being added towards the end. Addition of the lead salt is stated to produce an extremely sharp end-point. Any precipitate forming in the solution (urea nitrate) is disregarded. The interference of iron is overcome by adding sodium pyrophosphate before the thiocyanate. W. R. S.

Determination of Small Amounts of Nickel and Cobalt in Silicate Rocks. E. B. Sandell and R. W. Perlich. (*Ind. Eng. Chem., Anal. Ed.*, 1939, **11**, 309–311.)—*Nickel.*—The method was designed for rocks containing about 0·01 per cent. of nickel, a content too low to be determined by the gravimetric method of Harwood and Theobald (ANALYST, 1933, **58**, 673). The rock sample (0·25 g.) is obtained in solution by acid attack or fusion as necessary, 5 ml. of 10 per cent. sodium citrate solution are added, and the solution is rendered slightly ammoniacal; 2 ml. of 1 per cent. alcoholic dimethylglyoxime solution are added, and the liquid is extracted with two or three portions of chloroform, with vigorous shaking each time for half-a-minute. The chloroform extract containing the nickel glyoxime complex is washed once by shaking with 10 ml. of dilute (1 : 50) ammonia, and then shaken with two 4-ml. portions of 0·5 *N* hydrochloric acid. This decomposes the nickel glyoxime, and the nickel passes into the aqueous phase, in which it is determined by Rollet's method as follows:—The solution is diluted to 10 ml. with 0·5 *N* hydrochloric acid and 5 drops of saturated bromine water are added, followed by ammonia, drop by drop, until the bromine colour disappears, with 3 or 4 drops in excess; 0·5 ml. of 1 per cent. alcoholic dimethylglyoxime solution is added, and the colour developed compared in a Duboscq colorimeter with that formed by a standard nickel solution (less than 5γ per ml.) treated similarly, at the same time. Copper, cobalt, manganese, chromium and vanadium, in the amounts likely to be encountered in most igneous rocks, do not interfere; in test experiments, 100γ of cobalt gave a colour corresponding with 1·5γ of nickel; 100γ of copper treated gave no colour. It is stated that manganese in large quantities may cause trouble by oxidising nickel to the nickelic condition, in which it is not soluble in chloroform. *Cobalt.*—Extraction of the element with a carbon tetrachloride solution of dithizone from the ammoniacal citrate solution of the sample is employed. The carbon tetrachloride extract, which also contains the dithizonates of copper and other heavy metals, is evaporated to dryness, the

residue is ignited to destroy organic matter, and the residual oxides are dissolved in *aqua regia*. The solution is treated with stannous chloride to reduce copper to the cuprous condition, and the cobalt is determined colorimetrically by the addition of ammonium thiocyanate and acetone, essentially as described by Tomula (*Z. anal. Chem.*, **1931**, **83**, 6); alternatively, it may be determined by the thiocyanate-amyl alcohol method (O. Hackl, *Chem.-Ztg.*, 1922, **46**, 385). On a 1-g. sample of rock, as little as 0·0001 per cent. of cobalt may thus be determined.

S. G. C.

Separation of Cobalt from Manganese. J. G. Fairchild. (*Ind. Eng. Chem., Anal. Ed.*, 1939, **11**, 326–327.)—Manganese phosphate is precipitated in ammoniacal citrate solution, cobalt remaining dissolved. The solution containing manganese and cobalt, to which 2 g. of citric acid per 125 ml. have been added, is treated by the Gibbs method for the precipitation of manganese (*cf.* Hillebrand and Lundell, *Applied Inorganic Analysis*, 1929, p. 349). A small amount of cobalt tends to accompany the manganese in the precipitate, imparting a lilac colour. Re-precipitation of the manganese phosphate is advised. A simple method of correcting for co-precipitated cobalt is to determine it colorimetrically in the precipitate. The precipitate is dissolved in 20 ml. of dilute (1 : 1) hydrochloric acid, a few crystals of sodium sulphite are added, and the solution is evaporated to 10 ml.; the cobalt chloride colour is matched colorimetrically against a series of standards containing known amounts of cobalt treated similarly. The cobalt contained in the filtrate from the manganese phosphate is precipitated as sulphide at *p*H 5·2; the sulphide precipitate is afterwards converted into sulphate in the ordinary way, and the cobalt is weighed as such. Good results were obtained in test experiments with 2 to 50 mg. of cobalt in presence of 100 to 250 mg. of manganese. Alkaline earth metals and magnesium are precipitated as phosphates with pronounced co-precipitation of cobalt and should therefore be absent.

S. G. C.

Colorimetric Determination of Iron in Glass with the Use of Thioglycollic Acid. R. C. Chirnside and C. F. Pritchard. (*Trans. Soc. Glass Tech.*, 1939, **23**, 26–35.)—*Soda—Lime—Silica Glass.*—Ten ml. of hydrofluoric acid and 2 ml. of dilute (1 : 6) sulphuric acid are added to a 0·2-g. sample of glass, contained in an iron-free platinum crucible. The liquid is evaporated to dryness, the residue is ignited and fused with 0·5 g. of sodium carbonate, the cooled mass is dissolved in 10 ml. of 1 : 1 hydrochloric acid, and the solution is diluted to 100 ml. An aliquot portion is transferred to a 50-ml. Nessler tube, and 2 ml. of tartaric acid (10 per cent.), 2 ml. of dilute thioglycollic acid (1 : 10) and 10 ml. of ammonia (1 : 1) are added in that order. The purple-red colour of the iron-thioglycollic acid complex is matched against that of a similar solution (without sample), in another Nessler tube, titrated with standard iron solution (1 ml. = 0·00001 g. Fe_2O_3). A blank test on the reagents is advised. In absence of much alumina in the glass, fusion may be omitted: the solution in hydrofluoric-sulphuric acid is evaporated "to fumes," and the residue is dissolved, by heating, in water, with the addition of 5 ml. of conc. hydrochloric acid; the solution is diluted to 100 ml., and an aliquot portion is used for the colorimetric determination. *Alkali—Lead*

Oxide—Silica Glass.—A 0·2-g. sample is treated with mixed acids as before and evaporated "to fumes," 15 ml. of dilute sulphuric acid (1 : 10) are added, the liquid is boiled, and the lead sulphate is allowed to settle out, filtered off and washed, and the iron determination is carried out on the filtrate. *Coloured Glass.*—Cobalt, which may be present, interferes with the iron determination by giving a dark yellow-green colour, and requires to be separated by zinc oxide treatment. After acid attack and fusion of the residue as described above, the fused mass is dissolved in 10 ml. of dilute hydrochloric acid (1 : 1) and one drop of nitric acid is added to the hot solution to oxidise iron. The solution is diluted to 100 ml., and an aliquot part is neutralised with ammonia and just acidified with hydrochloric acid (pink colour with methyl red indicator); 100 mg. of pure zinc oxide are added, the liquid is well stirred and left for 10 minutes, and the precipitate is filtered off (No. 44 Whatman paper) and washed with water. The precipitate is dissolved from the paper with 10 ml. of warm dilute hydrochloric acid, the paper is washed, and iron is determined in the solution as previously described.

S. G. C.

Gravimetric Determination of Molybdenum as Trioxide. M. Straumanis and B. Ogrims. (*Z. anal. Chem.*, 1939, **117**, 30–47.)—The precipitation of molybdenum as sulphide (which is ignited to and weighed as trioxide) was reinvestigated. The authors find that the formation of molybdenum blue is avoided by dilution of the solution to less than 0·03 g. of MoO_3 per 100 ml. Further, the solution must be free from strong oxidising agents and also from phosphoric or arsenic acid. The acid concentration (hydrochloric, sulphuric, or nitric) may vary between 0·02 and 0·1 *N*, precipitation in a pressure-flask being unnecessary. A maximum concentration of hydrogen sulphide should be aimed at; hence a rapid stream of the gas is passed into the cold solution for 5 to 10 minutes. While the gas is passing the liquid is boiled for 10 minutes, allowed to cool, set aside for an hour, and filtered. The dried precipitate is cautiously ignited over a Bunsen burner until the paper has charred, then heated for 15 minutes in an electric furnace the bottom of which is kept at 500° C., and weighed as MoO_3.

W. R. S.

Separation of Tantalum from Niobium. H. Wirtz. (*Z. anal. Chem.* 1939, **117**, 6–9.)—A combination method is briefly described in which the oxidimetric determination of niobium is carried out on a weighed tannin precipitate containing the whole of the tantalum with very little niobium. This mode of working reduces the error incurred in the volumetric determination of niobium (*cf.* ANALYST, 1924, **49**, 215) to an almost negligible amount.

The weighed mixed oxides, which may contain titania (the quantity of which can be determined colorimetrically), are fused with potassium bisulphate, the melt is dissolved in ammonium oxalate solution, and the solution (diluted to 400 ml.) is treated with a few drops of bromophenol blue indicator and 1 to 2 ml. of strong sulphuric acid. The boiling solution is carefully treated with ammonia (1 : 3) until the yellow tint of the solution changes to a distinct purple. After addition of 10 g. of ammonium chloride, 2 per cent. tannin solution is added (quantity not stated), which precipitates the whole of the tantalum and titanium with only

0·01 to 0·02 g. of niobic oxide. After 20 to 30 minutes' boiling the precipitate is collected under slight suction, washed with hot 2 per cent. ammonium chloride solution, and ignited to constant weight. The oxide is fused with bisulphate, and the melt is dissolved in succinic acid solution (Metzger and Taylor, *J. Soc. Chem. Ind.*, 1909, **28**, 818). A measured amount of titanic sulphate solution is added to prevent hydrolytic dissociation of the niobium compound during treatment in the Jones reductor (ANALYST, 1922, **47**, 534), and the reduced solution is caught in ferric sulphate solution. The lower end of the reductor is immersed in the ferric solution. (The Nb_2O_5 is reduced to Nb_2O_3.) The volume of 0·1 *N* permanganate solution required to reoxidise the added titanium salt is ascertained by a blank test, from the result of which the volume of permanganate solution consumed by the titania in the ignited tannin precipitate can be calculated. The total volume of permanganate solution equivalent to titania is subtracted from the volume used in the titration; the difference is calculated to niobic oxide. The tantalic oxide in the ignited tannin precipitate is computed by subtracting the sum of titanic and niobic oxides found by titration from the weight of the precipitate.

W. R. S.

Tetraphenylarsonium Chloride as an Analytical Reagent. Determination of Rhenium. H. H. Willard and G. M. Smith. (*Ind. Eng. Chem., Anal. Ed.*, 1939, **11**, 305–306.)—To the hot solution (25 to 60 ml.) containing potassium perrhenate with sufficient sodium chloride to give a 0·5 *M* concentration in the final solution, a measured excess of tetraphenylarsonium chloride is added. The liquid is stirred and kept for several hours, preferably overnight. The white crystalline precipitate of tetraphenylarsonium perrhenate $[(C_6H_5)_4AsReO_4]$ is filtered off on a Gooch crucible, washed with ice-cold water, dried at 110° C. and weighed. Alternatively to gravimetric determination, the excess reagent in the filtrate may be determined potentiometrically with iodine (*id.*, 1939, **11**, 186; Abst., ANALYST, 1939, **64**, 452). The solution may be neutral, ammoniacal or acid up to about 4 *N* strength of acid. Nitrate, bromide, iodide and fluoride should not be present in more than small amounts. Permanganate, perchlorate, periodate, thiocyanate, mercury, tin, vanadyl, bismuth, lead and silver ions interfere. Cadmium and zinc are not detrimental if the chloride concentration is low. Tungstate, molybdate and metavanadate ions do not interfere with the precipitation of perrhenate in ammoniacal or tartrate-containing solutions. S. G. C.

Determination of Nitrogen in the Complex Cyanides of the Heavy Metals. V. E. Tischenko and A. M. Samsonifa. (*J. Ob. Chem.*, 1939, **9**, 160.)—For the determination of nitrogen in complex cyanides the sample is heated with dilute sulphuric or hydrochloric acid in 100-ml. sealed tubes for 1 hour in a furnace at 270° C., 25 ml. of dilute acid (1 : 4) being used. The nitrogen is subsequently determined as ammonia by distillation from alkaline solution. With dilute sulphuric acid the error varied from −0·04 to −0·31 per cent. in determinations on the salts $K_3Fe(CN)_6$, $K_2Hg(CN)_4$, $KAg(CN)_2$, $K_4Co(CN)_6$, $K_2Ni(CN)_4$, from 0·0912 to 0·5700 g. of salt being taken. With dilute hydrochloric acid under identical conditions, the error was smaller, ranging from −0·02 to −0·09 per cent. for weights of salts from 0·3290 to 0·8200 g. E. B. D.

Determination of Nitrogen in Stainless Steels. T. R. Cunningham and H. L. Hamner. (*Ind. Eng. Chem., Anal. Ed.*, 1939, **11**, 303–304.)—The method involves solution of the steel in acid and distillation of the ammonia; the recovery of nitrogen as ammonia from acid-insoluble nitrides of titanium, vanadium and tantalum is described. *Process.*—A 5-g. sample of the steel contained in a 300-ml. platinum dish with a well-fitting cover is dissolved as far as possible in 50 ml. of dilute (1 : 1) hydrochloric acid; 3 ml. of conc. hydrofluoric acid are added and the liquid is heated until all soluble material is dissolved [this treatment is not necessary if the sample dissolves completely in hydrochloric acid, and a platinum vessel is then not required]. The solution is transferred to a distilling apparatus, the distillation flask of which contains 500 ml. of water, 50 g. of sodium hydroxide, 20 g. of tartaric acid and some pieces of "spongy" zinc, the whole having been previously distilled for some time to remove possible traces of ammonia. The mixture is distilled, the distillate (200 ml.) being received in 0·02 *N* hydrochloric acid, and the excess of acid is back-titrated with standard alkali with the use of sodium alizarine sulphonate as indicator (end-point: disappearance of clear yellowish-green colour, or the first indication of brown); 1 ml. of 0·02 *N* acid $\equiv$ 0·00028 g. of nitrogen. Any insoluble residue obtained in the acid attack of the steel, which may contain nitride of vanadium, titanium, tantalum, or niobium, is filtered off, and the nitrogen determination is carried out on the filtrate. The filter-paper and residue are digested by heating in a Kjeldahl flask with 20 ml. of conc. sulphuric acid, 10 g. of potassium sulphate, and 1 g. of copper sulphate; 15 to 30 minutes' heating is allowed after the liquid has become clear. The sulphuric acid liquid is then distilled with alkali as for the main steel solution, the combined result giving total nitrogen in the steel. A blank determination, and the use of ammonia-free distilled water, are advised. Results agreeing well with the vacuum-fusion method were obtained on 18 : 8 chromium-nickel steel containing tantalum, and a 24 per cent. chromium steel containing titanium.

S. G. C.

Detection of Nitrites in Presence of Nitrates. E. Percs. (*Magyar Gyogyszerest Tars. Ert.*, 1939, **14**, 30–31; *J. Pharm. Belg.*, 1939, **21**, 403.)—The solution under examination is neutralised and treated with 5 drops of sulphuric acid and 1 drop of trypan red (see *Colour Index*, No. 438); in presence of nitrites the orange-red colour gradually changes to bright violet, the reaction being complete in 10 minutes. The test will detect 0·005 mg. of nitrite, and by using 0·5 ml. of the solution in a porcelain dish the sensitivity may be increased to 0·001 mg.

D. G. H.

Microchemical

Hygroscopic Substances in Microanalysis. I. Drying and Weighing Solids. II. Drying and Weighing Liquids. H. K. Alber. (*Mikrochem.*, 1938, **25**, 47–56; 167–181.)—I.—Hayman's definition of degrees of hygroscopicity (*Ind. Eng. Chem., Anal. Ed.*, 1932, **4**, 256; 1938, **10**, 55) is adopted, and a procedure is described for use mainly with type B, in which a 5-mg. sample increases in weight by more than 25γ in 5 minutes on the balance; type C must be handled without

coming into contact with the atmosphere, because even in a few seconds absorption of moisture would render the analytical results completely inaccurate. *Apparatus.*—A charging tube with ground cap is constructed from soft glass or Jena "Geräte glas," but it must not be of Pyrex, which tends to accumulate electrical charges. The tube, which is designed to hold 10 to 20 mg. of substance, weighs 1 to 5 g.; it is 2 cm. long and 0·5 cm. in diameter, and is provided with a long thin glass rod as a handle, 10 cm. long and 0·15 cm. in diameter, broadening to 0·3 cm. at the point of attachment to the base of the tube. The dead space in the ground-on cap should be as small as possible, and the joint, which must fit perfectly, should be 5 to 7 mm. in length. The tubes are supported in a small aluminium rack designed to fit in an Abderhalden drier, the cap of the charging tube being supported in the correct position for replacement, which is effected by pushing the handle of the tube. *Moisture determination.*—The charging tube is cleaned and dried for 15 minutes in the Abderhalden drier at the required temperature and pressure, dry air is then admitted, the drying agent ("anhydrone") is removed, and the cap is pressed into position with the aid of capillary forceps. The charging tube is left beside the balance for 20 minutes and then 5 minutes inside the balance with doors open and for 5 minutes with the doors closed. Shorter waiting periods are permissible when the drying temperature is below 100° C. A similar tube is used as a counterpoise and the weight is determined within 2γ. The sample is inserted by means of a 4-mm. spatula, and the tube is re-weighed after 10 minutes. The sample is then dried in the Abderhalden drier until constant weight is attained. Substances of types B and C may be successfully dried in this way; the method was tested with a large variety of substances, including nucleic acids, mercaptans, inorganic hydrates, etc. *Nitrogen (Dumas) determination on hygroscopic substances.*—The sample is dried in the charging tube, as described above, and transferred rapidly to a porcelain boat by opening the cap and tapping gently (a sufficient quantity of the sample for a duplicate analysis may be left in the tube—it does not absorb moisture when transference is rapid), and the tube is re-weighed. The sample in the boat is mixed with 40 to 80 mesh copper oxide in the combustion tube itself (see Milner and Sherman, *Ind. Eng. Chem., Anal. Ed.*, 1936, **8**, 427). *Carbon and hydrogen determination on hygroscopic substances.*—The sample is transferred from the charging tube to a platinum boat in a large desiccator containing P_2O_5, the cover is replaced on the desiccator and only removed while the boat is rapidly inserted into the side-arm combustion tube. The charging tube is capped and returned to the balance and re-weighed after about 15 minutes. This procedure is successful for type B compounds. For type C compounds the best method is to dry the sample in the combustion tube itself. When a number of analyses of one substance are required, satisfactory results may be obtained by making the determinations on undried samples and correcting for the moisture-content. The original sample is kept in closed charging tubes to prevent any change in moisture-content. It is assumed that the volatile matter is one substance only. When this is doubtful, a check analysis should be carried out on a dry sample.

II. Several procedures for drying hygroscopic liquids were tested; these vary according to the amount of sample available, contact with drying agent,

complete separation of drying medium and liquid, and specific properties of the liquids, such as vapour pressure and viscosity. The following procedure is for the automatic filling of weighing capillaries with almost complete exclusion of atmospheric moisture: An ordinary Pregl weighing capillary is weighed and inverted over a micro-centrifuge cone containing the liquid sample and the drying medium, and is held in position by means of aluminium wire (0·5 mm. diameter) so that the capillary tip does not reach the drying agent. The centrifuge tube is placed in a side-arm test-tube fitted with a stop-cock through the stopper. The test-tube is evacuated, bubbles escaping from the weighing capillary; drying is continued and then dry air is admitted through the stop-cock, so that dry liquid automatically rises up the capillary. If the tip has been immersed suitably, an air-bubble follows the liquid and acts as a seal. The weighing capillary is then cleaned, sealed and re-weighed. In experimental tests on both the milligram and decigram scale, losses of material (due to various factors) were investigated and found to amount to 0 to 40 per cent., the greatest loss occurring with ethyl ether.

J. W. M.

2-4-Dinitro-α-naphthol (Martius yellow) as a Reagent for Thallium and Cobalt. A. Martini. (*Mikrochem.*, 1938, **25**, 9–12.)—2-4-Dinitro-α-naphthol was tested as a reagent for alkaloids and for metals. Only amorphous precipitates are obtained with alkaloids and for the metals results were negative except with thallium and cobalt. The reagent is dissolved in pyridine and used when diluted with three volumes of water. A micro-drop (0·01 ml.) of a thallium nitrate solution (1 : 1000) is mixed on a slide with an equal-sized drop of reagent solution. Needle-shaped orange-yellow crystals form almost at once; *Limit of identification* 0·04γ, *concentration limit* 1:100,000. For the detection of such small amounts, it is advisable to evaporate the test solution to dryness before the addition of the reagent. Alternatively, the test may be applied to the detection of Martius yellow, a saturated solution of thallium nitrate being used as reagent. The test for cobalt is carried out by the same procedure as for thallium; *limit of identification* 0·5γ, *concentration limit* 1:10,000; an amorphous yellow precipitate is formed. Cobalt may be detected by this test in presence of 100 times the amount of nickel.

J. W. M.

Reviews

INDUSTRIAL CHEMISTRY. By WILLIAM THORNTON READ. Second Edition. Pp. ix + 605. New York: John Wiley & Sons, Inc.; London: Chapman & Hall. 1938. Price 25s. net.

In plan and arrangement this second edition closely resembles the original one, while the ground covered is also the same. The text, however, has been revised and brought up to date. The early chapters deal with general topics, such as the relation of chemistry to industry, the function of the chemist in industry, sources of information, economics, materials of construction, equipment and unit operations. These are written in an interesting manner and contain much useful information.

The remaining chapters deal with the manufacture of single products or groups of products, this grouping being based on chemical similarity, as may be seen from the following chapter headings: Sulphur and Sulphuric Acid; Fixed Nitrogen; Silicate Industries; Sodium, Calcium and Magnesium Compounds; Mixed Fertilisers; Hydrochloric Acid; Electrochemical Industries; Metallurgy; Petroleum; Animal and Vegetable Oils, Fats and Waxes; Carbohydrate Industries; Protein Industries; Rubber; Coal Products; Synthetic Drugs, Dyes and Resins; Explosives; Paints and Varnishes.

Such an arrangement leads to a certain amount of sectionalising of the information, so that, whilst the production of crude copper is dealt with in the chapter on metallurgy, the refining of the metal is placed in that on the electrochemical industries. Conversely, the fabrication of aluminium is described in the latter chapter, whilst its properties and alloys are listed in the former.

These chapters are, on the whole, well written and, although they do not pretend to deal exhaustively with each industry, they do cover a wide ground. Too wide, perhaps; for in certain places one gains the impression that in order to keep the size of the book within reasonable limits, the text has been rigidly condensed, with the result that occasionally it becomes vague or cryptic. Thus, in describing the Solvay process for the preparation of sodium carbonate, it is stated that ammoniacal brine is passed downwards through towers up which carbon dioxide is flowing, but no reason is given for the ensuing statement that the gas emerging from the top of these towers is mainly nitrogen. Again, in dealing with the recovery of iodine from Californian oil-well brines this sentence occurs: "the clarified brine is treated with just enough silver nitrate to precipitate the iodide as silver iodide from which it is liberated by steel scrap." As the text-book is intended mainly for the use of students, it would have been helpful if such statements had been amplified and more chemical equations provided.

The work is reasonably free from errors, except in the sections on synthetic drugs and dyes. Here they abound and are of a type one would hardly expect to find. Thus chloramine-T and dichloramine-T are stated to be ortho derivatives, while chlorazene (actually a synonym for chloramine-T) is given as the corresponding para compound. A number of the dyes listed have also been given incorrect, or, rather, incomplete graphic formulae, while acenaphthene and indanthrene are always referred to as acetnaphthene and inanthrene.

In spite of these blemishes the book contains much valuable information and provides a good one-volume survey of chemical industry. If it were equipped with references to original papers, articles or other text-books, it would be valuable to an even wider range of readers than students. G. R. DAVIES

MODERN CEREAL CHEMISTRY. Third Edition, Revised and Enlarged. By D. W. KENT-JONES. Pp. vii + 720. Liverpool: The Northern Publishing Co., Ltd. 1939. Price 30s.

The new edition of Modern Cereal Chemistry is approximately twice the size of its predecessor and contains four new chapters on:—Dough Testing Machines; Flour for purposes other than Breadmaking; Cereal and Balanced Rations for Livestock; The Microbiology of Cereals (A. J. Amos).

All the old chapters have been revised in the light of recent advances, not only in cereal chemistry, but also in other branches of science which affect the milling and baking industries. More than one hundred pages are now devoted to methods of analysis.

The results of most investigations into problems of cereal chemistry are described in detail, together with the author's own comments; the text—as in previous editions—is pleasingly full of references to cognate literature, and the book contains much useful information accumulated by the author during his years of wide experience in the milling industry. The chapter on moisture in wheat deals clearly and concisely with a subject which must have proved at some time or another a problem to many analysts.

The new chapters on dough-testing machines and the microbiology of cereals are very welcome, in that they provide, particularly on the subject of dough-testing machines, information not readily attainable in the literature.

Some points of criticism, however, must be raised on the chapter on "Cereal and Balanced Rations for Livestock." In this chapter some of the definitions of feeding stuffs are misleading. Maize gluten feed, for instance, is prepared from maize in the production of maize starch and not, as stated in the text, in the manufacture of molasses.

In the section on oil-cakes it should be more clearly indicated that such cakes should be free from contamination with the poisonous castor-oil seed. The limit of castor-oil seed content as indicated by 0·005 per cent. of castor-oil seed husk, as given in the text, is sometimes fixed in commercial contracts as a limit beyond which the purchaser of a cargo or parcel may be entitled to claim "total rejection"; but the danger of this contamination is further recognised in such contracts by the infliction, on the vendor, of a money penalty per ton on a sliding scale for the presence of lesser quantities of castor husk down to even 0·001 per cent.

In connection with the author's reference to hydrocyanic acid and linseed cake, it is not made clear that the function of boiling water, which is very properly emphasised in the preparation of linseed gruel for calves, is not to expel hydrocyanic acid, but to kill or cripple the enzyme which may liberate it from the glycoside normally present.

The expression "Oat Meal" is used by the author to indicate ground oats. Ground oats are usually commercially described as such, not as "Oat Meal."

Oat Meal is meal made from de-husked oats, as for human consumption. It is true that ground barley is always called "Barley Meal," but the analogous expression "Oat Meal" is not used for ground oats.

These minor criticisms, however, apply only to a portion of the book which may be regarded as supplementary to its main contents, for the re-issue and expansion of which the author deserves warm gratitude. J. HUBERT HAMENCE

A TEXTBOOK OF PHARMACOGNOSY. By T. C. DENSTON, B.Pharm., A.I.C., Ph.C. Third Edition. Pp. xvi + 582. London: Sir Isaac Pitman & Sons, Ltd. 1939. Price 20s. net.

The publication of a third edition of a textbook on any subject is testimony that it meets the requirements of a number of purchasers, but it also gives the author an opportunity to extend, amplify, and improve the original copy. In this instance, the third edition has permitted a large amount of revision, and the inclusion of many fresh chapters, which indicate the increasing scope of the subject.

Materia Medica has always been correlated with botany because so many of the substances employed in medicine are of vegetable origin, but it is only during recent years that *pharmacognosy* has attained a definite place in a university syllabus. The modern textbook deals with something more than the botanical name, habitat and chief constituents of the important drugs, and some of the sections in this book possess a very definite educational value.

A chapter on drug constituents and their applications in pharmaceutics, contains, in the space of some sixty pages, the real essentials of pharmacognosy, and the section dealing with vitamins is as comprehensive as any short account of these relatively new substances can be while new facts are constantly being added to our present knowledge.

The section dealing with the cultivation, collection, stabilisation and preservation of drugs will be of service to students and others approaching the subject with an open mind. Such information can only be obtained by practical experience, and it has been very comprehensively dealt with in the space available.

The chapters devoted to the description of leaves, flowers, seeds, fruits, plants and herbs, barks, roots and rhizomes, are framed in the manner usual for a textbook on materia medica, and are fully illustrated and complete in detail, although some of the drawings leave out certain undefinable characteristics which only practical handling of the particular drugs can impart to a buyer or student. A photographic illustration of digitalis on page 119 affords a perfect picture of all the details by which the plant can be identified, but the drawing on page 124, and those on 127, would be of no practical assistance to a novice hoping to identify any adulterants. This lack of detail in the sketches is more apparent in the representations of rhubarb, jalap, and ipecacuanha, and suggests that the artist should pay a visit to the drug warehouses of the Port of London Authority to seek the familiarity which only comes when handling drugs in bulk. In contrast to these, the drawings on figures 85 and 86 depict what are typical structures in such a way as to make them recognisable when found under a microscope.

The sections dealing with oils, fats, and waxes, and those on volatile oils, are

comprehensive, and include the official tests of the British Pharmacopoeia and some useful illustrations. A novel section consists of a series of maps showing some of the geographical features of parts of the world too well-known to need depicting, but a list of drugs and the countries where they are produced may be of service to those needing such information.

This is essentially a book for students, but the information is reliable and well arranged, and the recent additions and improvements will make it a useful book of reference. The line-drawing illustrations are contributed by Mr. M. Riley, and the new plates dealing with fibres, plant hairs and insect pests are from the hands of Miss Rosemary Butterfield, B.Pharm. C. EDWARD SAGE

THE CHEMISTRY OF MILK. By W. L. DAVIES, Ph.D., D.Sc., F.I.C. Second Edition. Pp. xiv + 534. London: Chapman & Hall. 1939. Price 25s.

That a second edition of this book has been called for is proof that it fills a useful position in the library of the dairy chemist. The revision has been, on the whole, well and carefully done, though there are a few typographical errors.

Some subjects are treated in greater detail than is necessary in a book which is not a practical treatise; for example, the preparation of pure casein (p. 146) and the description of the Kay phosphatase test (p. 363). On the other hand, there are certain curious omissions; for instance, there is no reference to the Lampitt and Hughes method for the accurate determination of the solubility of milk powders, which is still the best that has been published, nor to those useful weapons in the investigation of fat oxidation, the peroxide and Kreis tests; while the important effect of dilution on the titratable "acidity" of milk is mentioned, the equally important effect of the amount of indicator (to which attention was directed by Pizer) has not been mentioned. Dr. Davies has done a distinct service in directing attention to the relative inaccuracy of the figures for total solids based on lactometer readings; it would, however, be interesting to learn his authority for the statement that differences of +0·5 to −0·4 are possible. In the paper by Bartlett, Golding and Wagstaff referred to, the maximum error from actual determinations is shown to be about +0·28 to −0·10—figures that agree closely with those obtained in the writer's laboratory after allowance had been made for errors due to the physical condition of the fat, and in reading the lactometer and Gerber tubes; there is also considerable doubt whether at any time there is a significant change in the specific gravity of the solids-not-fat.

The author is less happy on the technological than the scientific side, which is to be expected, as years of experience are the only method of obtaining competence in any branch of the subject. It is not intended, however, to dissect the book paragraph by paragraph, but it is desirable to correct the statement on p. 428, referring to atmospheric roller-dried powder, that "when the product is intended for human consumption," "this process is disadvantageous," because of its lower solubility. This is not borne out by the fact that practically all maternity centres use these powders for infant feeding.

Despite these criticisms the book is a useful compilation "full of meat," and the tables have proved particularly valuable. If any student requires subjects for research in dairy chemistry, he has only to note how frequently subjects with the words "may be," "possibly," and "probably" occur. E. B. ANDERSON

WOOD PULP. By JULIUS GRANT, Ph.D., F.I.C. Pp. xi + 209. Leiden: Chronica Botanica Co.; London: Wm. Dawson & Sons, Ltd. 1938. Price 7 guilders (about 16s.).

This volume, of some 200 pages, is one of a new series of plant science books published in English by Chronica Botanica of Leiden, Holland. The author, whose experience in paper-making and allied subjects is well-known, states in his preface that while the series of publications is intended to cater especially for those interested in plant science, "it has been felt that wood pulp and the uses to which it is put has now become of such widespread interest and importance, that the opportunity should be taken to produce a work which will also appeal to students of other sciences as well as to general readers." In the present volume the author has definitely fulfilled this object.

The book gives a complete account of wood pulp in 20 short chapters, each one of which deals briefly and clearly with some necessary aspect of the subject. The first four chapters will be of particular interest to the botanist as well as to the general reader. They deal with the nature of pulp, the structure and growth of wood, and the distribution of wood pulp forests. A very interesting chapter on the historical development of the art of paper-making and the use of wood pulp—a subject the author has made particularly his own—follows. The structure of cellulose and wood fibres and the identification of the different types of wood used in pulping are then discussed.

The more technical side—the preparation of the wood, the sulphite, soda, and sulphate processes—is next considered, and here the simple chemistry, for example, of the sulphate process, is fully explained, so that the reader with little chemistry can follow the various stages. A sufficient account of the working of each process is given.

Bleaching is then discussed, and the modern use of chlorine to remove lignin prior to treatment with hypochlorite is explained. The mechanical purification of pulp and the utilisation of by-products are included, and final chapters deal with testing by physical and chemical means, and valuation of the pulps produced. The analytical processes given are, in many instances, those specified by The Technical Association of the Pulp and Paper Makers' Association of America. A few typical analyses would have helped the general reader of this section.

The uses of pulp for paper, rayon and many other purposes are fully described, and a few words as to paper and wood pulp in the future conclude the volume, which can be recommended as a well-written and most interesting treatment of the subject.

C. DORÉE

HAIR-DYES AND HAIR-DYEING CHEMISTRY AND TECHNIQUE. By H. STANLEY REDGROVE, B.Sc., F.I.C., and J. BARI-WOOLLSS. Pp. 214. London: William Heinemann, Ltd. 1939. Price 10s. 6d.

This is the third edition of *"Blonde or Brunette ?"* first published by Redgrove and (the late) G. Foan in 1929, and, like the original edition, has two distinctive merits: one that it is a collaboration between a skilled chemist and a practical

hairdresser, and the other that it is the only book in our language dealing comprehensively with this subject. Both the artist and the chemist may be included in the quotation from Thomas Moore:

> While some bring leaves of henna, to imbue
> The fingers' ends with a bright roseate hue,
> . . . . . .
> . . . . . .
> And others mix the Kohol's jetty dye
> To give that long, dark languish to the eye.

The artist, in the shape of the practical hairdresser, has reigned in this field since the days of Enoch, and it is only of late years, with the advent of the new types of dye, that the subject has begun to receive from chemists the attention it deserves. The manufacture of hair-dyes and the technique of hair-dyeing are now quite an important branch of industry, and we are indebted to the authors for collecting and sifting so much information; they have given a connected scientific exposition of the subject in simple language.

After a general survey of the structure and properties of hair there follow chapters on bleaches (of which there are many), kohl, lead and silver dyes, tannin rasticks, walnut, henna, rengs, chamomile, and the wide field of the diamine class. The chemistry of each is explained, and practical applications are outlined. Then follow some ten chapters on the practical technique of hair-dyeing, and finally a section on grey hair and a bibliography.

Chemists will, of course, be mainly interested in Mr. Redgrove's part, which deals with the composition of various hair-dyes and mixtures. This section is excellent, the information is up-to-date and, so far as the reviewer can test it, accurate. It is, of course, inevitable that *p*-phenylenediamine should be discussed at some length; it is indeed a versatile compound, and if it does cause dermatitis sometimes to the few, it brings brightness to the million by the variety of shades that it can be induced to afford. Many are the ingenious devices which have been put forward to remove its irritant properties, but they are at best only partly effective; yet this substance remains the best all-round hair-dye. Its reactions and oxidation products in the presence of poly-phenols still offer an almost unexplored field for research.

The book as a whole is well written and very informative; it is likely to be most useful to all of us who have occasion to analyse hair-dyes or similar products and is full of interest, too, to the general reader. We warmly commend it.

H. E. Cox